Ecology, Population Genetics and Risk Assessment of the Exotic Mosquitofish, *Gambusia holbrooki*, in Tasmania

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Declaration of originality

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Abstract

The Poeciliid fish, *Gambusia holbrooki*, a native of North America, has been deliberately introduced into many countries for the purpose of mosquito control. Extensively introduced on mainland Australia since the early 1900's, the fish was absent from the island state of Tasmania until it was illegally introduced in 1992. Since that time, the fish has spread throughout the Tamar Estuary in northern Tasmania. Surveys conducted from 2004-2006 revealed that the fish have spread naturally at the modest rate of less than 2 km per year. Populations showed synchronous birth of live young occurred in mid-November each year, and mean abundance was highest in the summer months (~60 fish/0.5 m^2). *Gambusia* fed predominantly on micro-crustaceans throughout the year, while mosquitoes and amphibians made up a very minor proportion of the diet (<2% in any season). *Gambusia* appeared to negatively impact tadpole communities with abundance of tadpoles significantly increasing following eradication of the fish.

Population genetic analysis using microsatellite markers revealed that Australian *Gambusia* populations are characterized by low diversity and Tasmanian *Gambusia* were possibly derived from southeast Queensland. Although significant differentiation existed among most populations, Tasmanian *Gambusia* populations were generally genetically similar, indicating a single introduction event. The genetic pattern of relationships among Tamar populations indicated that the site, TIWR, is driving the spread of *Gambusia* throughout the estuary.

In May 2005, an attempt was made to eradicate *Gambusia* from two enclosed water bodies in the Tamar region. Water was pumped from both sites prior to application of hydrated lime ($Ca(OH)_2$). Both eradications proved unsuccessful, and populations were monitored and samples taken to assess the population recovery and genetic consequences

of the eradication attempts. The Riverside population recovered quickest with live fish sighted 17 days after the initial lime treatment compared to 8 months at LD2. Estimates of effective population size (N_e) indicated that less than ten individuals survived the eradication attempt at Riverside [6 (95% CI=2-14)], and less than five at LD2 [1 (95% CI=0-3)]. Allele frequencies varied significantly at both sites following the eradication attempt, and some alleles were not detected post-eradication. However, there was no statistically significant loss of allelic diversity at either of the sites. The rapid recovery of both populations combined with the maintenance of genetic diversity and minimal changes in allele frequencies, indicate that *Gambusia* are particularly resistant to the negative genetic effects of bottleneck events that dramatically decrease population size.

A site-based risk assessment (RA) protocol was developed from a survey of 27 sites within and around the current distribution of *Gambusia* in the Tamar Estuary. The results of the RA generally reflected the actual situation on the ground with only 3 of the 27 sites returning a risk score of ≥30 (all currently support large *Gambusia* populations). Over 85% of sites returned a risk score from 1-20, with all sites where the fish has never been encountered or failed to establish returning scores of ≤20. The RA could be utilized by managers of *Gambusia* in Tasmania to rapidly assess new survey sites in the Tamar region and to guide monitoring considerations in the future.

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CHAPTER 1:

Introduction

1.1 Background

The transfer, introduction and establishment of non-indigenous species (NIS) has become a major problem in many countries throughout the world due to increased trade and globalization (Ricciardi and Rasmussen 1998). Between 10 and 30% of these species become pests, causing environmental and economic damage in the receiving environment (Williamson and Fitter 1996; Pimentel *et al.* 2000). In the United States, with approximately 50,000 NIS species, environmental and economic losses total ~$137 billion per year (Pimentel *et al.* 2000). In Australia, one study conservatively estimated that 10 terrestrial vertebrate pest species cost at least $369.9 million per year, although complete estimates are not available (McLeod 2004). The only freshwater vertebrate pest for which there are estimates is the European carp, *Cyprinus carpio*, which causes economic losses of at least $4 million per annum in Australia (McLeod 2004). This figure however, does not include losses due to environmental impacts which have been estimated to be approximately $11.8 million per year (McLeod 2004). Other aquatic vertebrates that have become invasive in Australia and are having a negative impact on native species and other aquatic species include: eastern *Gambusia* or mosquitofish, *Gambusia holbrooki*; redfin perch, *Perca fluviatilis*; rainbow and brown trout, *Oncorhynchus mykiss* and *Salmo trutta*; tench, *Tinca tinca*; swordtail, *Xiphophorus hellerii*; tilapia, *Oreochromis mossambicus*; and weatherloach *Misgurnus anguillicaudatus* (Agtrans Research 2005).

The process of invasion by NIS generally follows four main phases; transportation, release, establishment and spread (Kolar and Lodge 2001). To begin the invasion process, a species must be moved outside its native range by a transport pathway. From the time a species is released it interacts with the invaded ecosystem. These interactions, together

with other factors, determine whether the invading species will become established. Many NIS fail to establish, some will remain localized near the point of introduction, while others spread widely and become invasive (Kolar and Lodge 2001). Often a period of little or no range expansion will occur after the initial introduction, termed a "lag phase" (Crooks and Soule 1999; Allendorf and Lundquist 2003). During this lag phase, adaptation and/or hybridization can occur and negative impacts (environmental and economic) are generally not reported (Crooks and Soule 1999; Allendorf and Lundquist 2003).

Invasions of marine, estuarine and terrestrial ecosystems generally follow the pattern described above. However, several comparative studies have shown that NIS tend to spread more rapidly in terrestrial than in marine systems (Grosholz 1996). This is surprising given that there is a high potential for long distance dispersal of larval phases in marine systems. There are several possible explanations for this pattern. It may be due to taxonomic differences between the NIS compared rather than habitat differences, or it could be that invasions in marine systems are simply more variable (Grosholz 1996). It is certainly true that there is a far more extensive literature base documenting invasions and impacts of NIS in terrestrial and freshwater habitats than in coastal marine systems (Grosholz and Ruiz 1995). In coastal marine system when species are identified as NIS, information about their source, time of arrival, rate of spread, current distribution and impacts is often poor (Grosholz and Ruiz 1995).

This research focuses entirely on the mosquitofish, *Gambusia holbrooki*, and its recent invasion of estuarine and freshwater habitats in northern Tasmania, Australia. The term mosquitofish collectively includes two fish species of the family Poeciliidae, *Gambusia holbrooki* and *Gambusia affinis* (Krumholz 1948; Rivas 1963; Wooten *et al.* 1988). Mosquitofish are native to southern North America and are small relatively non-descript

fish reaching a maximum size of approximately 6 cm (Allen *et al.* 2002). *Gambusia* are sexually dimorphic, and sexes can be distinguished externally. Females are generally larger and have a dark peritoneal area on the side of the body (Krumholz 1948; Allen *et al.* 2002). Adult males have an external insemination organ or gonopodium that is used to fertilize females internally (Meffe 1992). *Gambusia* are ovoviviparous, capable of producing several (4-9) clutches of 1 to >100 live free swimming young per breeding season (Pyke 2005). They are able to survive in a wide range of physical conditions: water temperatures from 1°C – 40°C, salinities from fresh to sea-water, and dissolved oxygen levels as low as 0.28 mgL^{-1} (Pyke 2005). Similarly, they are highly resistant to the effects of toxins such as rotenone and insecticides when compared to other fish (Pyke 2005).

Gambusia holbrooki and *G. affinis* are collectively the most widespread fish in the world and are be found on all continents except Antarctica (Krumholz 1948; Lloyd and Tomasov 1985; Arthington and Lloyd 1989). Their widespread distribution is primarily due to their popularity as an aquarium fish and their perceived benefits as mosquito control agents (Wilson 1960; Green and Imber 1977; Courtenay and Meffe 1989). In Australia, *G. holbrooki* were first introduced in the early 1900's (Wilson 1960; Clunie *et al.* 2002). Since then, their distribution throughout the country has been facilitated by the defence forces and municipal councils in an effort to control mosquitoes and mosquito-borne diseases (Wilson 1960; Clunie *et al.* 2002). Although *Gambusia* are generalist feeders, they do not preferentially prey on mosquito larvae or adults, and their effectiveness as mosquito control agents is now generally considered to be minimal (Lloyd 1986; Lloyd 1990; Blaustein 1992; Garcia-Berthou 1999). In addition to not fulfilling their intended role as mosquito control agents, *Gambusia* are considered a pest species in most of the recipient

Figure 1.1. Pregnant female *Gambusia holbrooki* a), shown here with several common Tasmanian tadpoles. b) *Litoria ewingii* (brown tree frog), c) *Limnodynastes dumerilii insularis* (Pobblebonk or Banjo frog) and d) *Crinia signifera* (common froglet), and e) juvenile *Gambusia holbrooki* (<1week old).

environments due to their negative impacts on native aquatic species (Courtenay and Meffe 1989; NSW Parks & Wildlife Service 2003). Their establishment success has been primarily attributed to their capacity for rapid reproduction, opportunistic feeding and their ability to survive in a wide range of environmental conditions (Arthington and Lloyd 1989; Courtenay and Meffe 1989; Pyke 2005). Regardless of the reasons, they have been responsible for a range of negative impacts on native species. For example, *Gambusia* will actively compete for resources with adult native fishes by attacking and fin-nipping, and will prey on the eggs and juveniles of native fish species (Howe *et al.* 1997; Ivanstoff and Aarn 1999). Furthermore, *Gambusia* are also responsible for large scale declines in some mainland Australian frog species due to their predation on eggs and tadpoles (Webb and Joss 1997; Komak and Crossland 2000; Hamer *et al.* 2002). Several studies have shown that *Gambusia* actively compete with native fish and frog species, and that they may be responsible for the decline/localised extinction of at least 35 species worldwide (Lloyd 1990; Lawler *et al.* 1999; Kats and Ferrer 2003). At the ecosystem level, *Gambusia* can reduce water quality by intensive feeding on zooplankton, which in turn promotes algal blooms (Hurlbert and Mulla 1981). Reduction in water quality can, in turn, lead to exclusion of species that require pristine environmental conditions (Lloyd, 1990).

1.2 *Gambusia* in Tasmania

Although present in all Australian mainland States and the ACT (except NT), *Gambusia* were excluded from the island state of Tasmania until 1992, when a small number of *G. holbrooki* (approx. 50) were illegally introduced into a small farm dam (LD1) north of Launceston in northern Tasmania (see Fig. 2.1). The State government Inland Fisheries

Service (*hereafter* IFS) received information that a private landowner had illegally transported the species from his property in southeast Queensland to a northern Tasmanian property he also owned. The IFS carried out several eradication attempts in the area and *Gambusia* were thought to have been restricted to two small private dams at Legana (IFS *unpublished data*). However, in 2000, *Gambusia* were discovered in the Tamar Island Wetlands Reserve (TIWR), approximately eight kilometres south-west (upstream) of the original introduction site (Keane 2002; Keane and Neira 2004). Subsequent investigations and contact with the person responsible for the Legana introduction revealed that *G. holbrooki* had been introduced into the TIWR at the time of the initial introduction at Legana (IFS *unpublished data*).

The *Gambusia* Management Committee (comprising individuals representing a range of stakeholder groups) was formed following the discovery of *Gambusia* at TIWR. The goal of the *Gambusia* Management Committee (GMC) was to coordinate management of the fish and to prioritize research, eradication and control efforts in the Tamar region. Following consultation with GMC, a conceptual model of the invasion of *Gambusia* in the Tamar region was constructed (Fig. 1.2). The conceptual model, although simplified, helps identify sources and stressors of the invasion, and provides a framework for literature review, research directions and discussion with stakeholders (Landis 2003). Determination of the extent of spread and the possible impacts of *Gambusia* on Tasmanian native species was considered to be the primary research objective and was therefore a core aim of this study. However, it was clear that any research contributing to knowledge concerning the pathways identified in the conceptual model would be beneficial to our overall understanding of the *Gambusia* invasion of the Tamar Estuary. Therefore, this study provides additional insights into the dispersal and establishment of *Gambusia* in the early

phase of the invasion process, the genetic effects of attempted eradication and provides a useful management tool for identifying sites most at risk of *Gambusia* invasion.

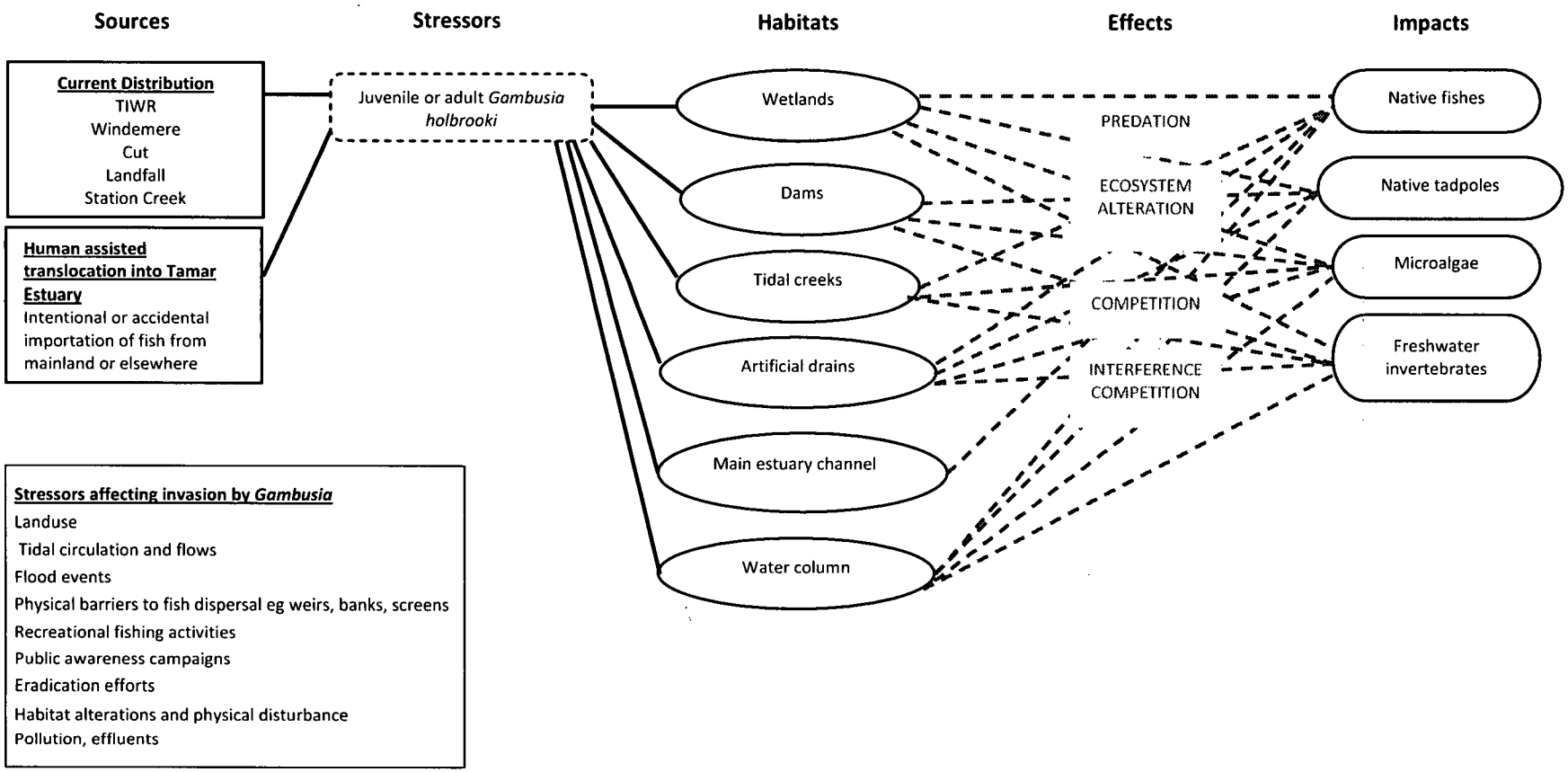

Figure 1.2. Conceptual model representing the *Gambusia holbrooki* invasion of the Tamar Estuary.

1.3 Aims

In this thesis, the progress of the *Gambusia* invasion of Tasmania is documented, whilst new research with applied significance and relevance to future managers of the fish in the state is also presented. The main aim of this thesis was to assess the status of *Gambusia holbrooki* in Tasmania. This issue was addressed by research in the following key areas:

1) Determine the ecology and distribution of *Gambusia* in the Tamar Estuary region. In particular, examining aspects of life history such as population size- structure, reproduction, and diet.
2) Assess of the effect of *Gambusia* on tadpole/frog communities.
3) Examine *Gambusia* population genetic diversity and differentiation using microsatellite DNA analysis to investigate whether the anecdotal evidence of a Queensland source for Tasmanian *Gambusia* is supported.
4) Examine *Gambusia* population recovery and genetic diversity after unsuccessful eradication.
5) Develop a habitat based risk assessment model to predict which sites are most at risk from *Gambusia* invasion and establishment.

1.4 Chapter summaries

This thesis consists of four data chapters (Chapters 2-5), presented as separate

manuscripts. This arrangement leads to replication in some parts of the text. However, some referencing of other chapters has been added to the text to assist the reader in linking the chapters. Chapter 6 is a summary of the key outcomes of this research, its applied significance, and avenues for future research. Outlined below is a summary of the aims of each data chapter, combined with a brief description of the data used to address these aims.

Chapter 2: Aspects of the ecology of the recently introduced mosquitofish, *Gambusia holbrooki*, in Tasmania.

The main aim of this chapter was to describe the ecology and distribution of *Gambusia* in the Tamar estuary as it was unclear how far the species had spread since their initial introduction and if there were negative impacts on Tasmanian native species. The extent and rate of spread of *Gambusia holbrooki* in the Tamar Estuary is described in addition to an examination of the temporal patterns of abundance, diet, and reproduction in the largest established Tamar population of the fish. The likely impact of *Gambusia* on Tasmanian native frog populations is assessed by comparison of tadpole communities in the presence and absence of *Gambusia.* Implications of further range expansion by *Gambusia* throughout Tasmania are discussed.

Chapter 3: Genetic diversity and population differentiation of the non-indigenous fish, *Gambusia holbrooki*, in Australia.

In Chapter 3, patterns of diversity and population genetic structure of Tamar

Estuary and mainland Australian *Gambusia* populations are examined using hypervariable microsatellite DNA loci. The relatively recent and well documented introduction of *Gambusia* into Tasmania provided a unique opportunity to examine the population diversity and genetic structure of a non-indigenous species in the early stages of establishment and dispersal. Genetic structure and diversity of several mainland populations of *Gambusia* are compared in order to identify the likely source of the Tasmanian introduction and to assess the effect of the long history of human mediated dispersal on the genetic diversity of Australian *Gambusia* populations. Genetic differentiation among Tasmanian *Gambusia* populations is evaluated to identify core or source populations driving the dispersal of *Gambusia* throughout the Tamar Estuary.

Chapter 4: The effects of attempted eradication on genetic diversity of two populations of *Gambusia holbrooki.*

The broad aim of this chapter was to examine the effect(s) of a severe reduction in population size (bottleneck) on populations of *Gambusia holbrooki. Gambusia* eradications were attempted at two small dams in the Tamar region in an effort to prevent further spread of the species in the region. Complete eradication of *Gambusia* was not achieved at either site. Population recovery and the effect of eradication attempts on genetic diversity are assessed.

Chapter 5: Site-based risk assessment as a tool for evaluating risk of *Gambusia* invasion of the Tamar Estuary.

The main aim of this chapter was to develop a risk assessment protocol that could be utilized to identify sites in the Tamar Estuary at high risk of *Gambusia* invasion. The risk assessment was constructed using information during this study and supplemented by the data available in published literature. The risk assessment utilizes some of the vast amount of research effort that has focused on *Gambusia* over the past several decades in order to produce a management tool that is effective in the field and can be applied to other circumstances.

1.5 References

Agtrans Research (2005) 'Review of progress on invasive species.' Department of Environment and Heritage, Canberra, Australia.

Allen G. R., Midgley S. H. and Allen M. (2002) 'Field guide to the freshwater fishes of Australia.' 394 pgs. (Western Australian Museum: Perth).

Allendorf F. W. and Lundquist L. L. (2003) Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Arthington A. H. and Lloyd L. N. (1989) Introduced Poeciliids in Australia and New Zealand. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 333-348. (Prentice Hall: New Jersey).

Blaustein L. (1992) Larvivorous fishes fail to control mosquitoes in experimental rice plots. *Hydrobiologia* **232**, 219-232.

Clunie P., Stuart I., *et al.* (2002) 'A risk assessment of the impacts of pest species in the riverine environment in the Murray-Darling Basin.' Department of Natural Resources and Environment, Arthur Rylah Institute for environmental research, Project R2006, Heidelberg, Victoria.

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Crooks J. A. and Soule M. E. (1999) Lag times in population explosions of invasive species: causes and implications. In 'Invasive species and biodiversity management'. (Eds OT Sandlund, PJ Schei and A Viken) pp. 103-126. (Kluwer Academic Publishers).

Garcia-Berthou E. (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* **55**, 135-147.

Green M. V. and Imber C. F. (1977) Applicability of *Gambusia affinis* to urban mosquito problems in Burlington County, New Jersey. *Mosquito News* **37**, 383-385.

Grosholz E. D. (1996) Contrasting rates of spread for introduced species in terrestrial and marine systems. *Ecology* **77**, 1680-1686.

Grosholz E. D. and Ruiz G. M. (1995) Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. *Marine Biology* **122**, 239-247.

Hamer A. J., Lane S. J. and Mahony M. J. (2002) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* **132**, 445-452.

Howe E., Howe C., Lim R. and Burchett M. (1997) Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine & Freshwater Research* **48**, 425-434.

Hurlbert S. H. and Mulla M. S. (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* **83**, 125-151.

Ivanstoff W. and Aarn (1999) Detection of predation on Australian native fishes by *Gambusia holbrooki. Marine and Freshwater Research* **50**, 467-468.

Kats L. B. and Ferrer R. P. (2003) Alien predators and amphibian decline: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9**, 99-110.

Keane J. P. (2002) Population structure, reproductive onset and temperature tolerance of the mosquitofish *Gambusia holbrooki* in northern Tasmania. Australian Maritime College.

Keane J. P. and Neira F. J. (2004) First record of mosquitofish, *Gambusia holbrooki*, in Tasmania: stock structure and reproductive biology. *New Zealand Journal of Marine and Freshwater Research* **38**, 857-868.

Kolar C. S. and Lodge D. M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* **16**, 199-204.

Komak S. and Crossland R. (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* **27**, 185-189.

Krumholz L. A. (1948) Reproduction in the western mosquitofish *Gambusia affinis affinis* (Baird and Girard) and its use in mosquito control. *Ecological Monographs* **18**, 1-43.

Landis W. G. (2003) Ecological risk assessment conceptual model formulation for nonindigenous species. *Risk Analysis* **24**, 847-858.

Lawler S. P., Dritz D., Strange T. and Holyoak M. (1999) Effects of Introduced Mosquitofish and Bullfrogs on the Threatened California Red-Legged Frog. *Conservation Biology* **13**, 613-622.

Lloyd L. (1990) Native fishes as alternative to the exotic fish, *Gambusia*, for insect control. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 115-122. (Australian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. N. (1986) An alternative to insect control by "mosquitofish", *Gambusia affinis*. In '4th Australian arbovirus symposium'. Brisbane. (Eds TD George, BH Kay and J Blok). (Arbovirus research in Australia).

Lloyd L. N. and Tomasov J. F. (1985) Taxonomic status of the mosquitofish, *Gambusia affinis* (Poeciliidae), in Australia. *Aust.J.Mar.Freshwat.Res.* **36**, 447-451.

McLeod R. (2004) 'Counting the cost: Impact of invasive animals in Australia 2004.' Cooperative Research Centre for pest animal control, Canberra.

Meffe G. K. (1992) Plasticity in life-history characters in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in response to thermal stress. *Copeia* **1**, 94-102.

N. S. W. Parks & Wildlife Service (2003) 'Predation by *Gambusia holbrooki* - The plague minnow.' National Parks and Wildlife Service, Hurstville, NSW.

Pimentel D., Lach L., Zuniga R. and Morrison D. (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience* **50**, 53-65.

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* **15**, 339-365.

Ricciardi A. and Rasmussen J. B. (1998) Predicting the identity and impact of future biological invaders: A priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1759-1765.

Rivas L. R. (1963) Subgenera and species groups in the poeciliid fish genus *Gambusia* Poey. *Copeia* **1963**, 331-347.

Webb C. and Joss J. (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist* **30**, 316-326.

Williamson M. and Fitter A. (1996) The varying success of invaders. *Ecology* **77**, 1661-1666.

Wilson F. (1960) 'A review of the biological control of insects and weeds in Australia and Australian New Guinea.' 102 pgs. (Commonwealth Agricultural Bureaux: Bucks, England).

Wooten M. C., Scribner K. T. and Smith M. H. (1988) Genetic variability and systematics of *Gambusia* in the southeastern United States. *Copeia* **2**, 283-289.

CHAPTER 2:

Aspects of the ecology of the recently introduced mosquitofish, *Gambusia holbrooki,* in Tasmania

2.1 Introduction

Mosquitofish, which include *Gambusia holbrooki* and *G. affinis*, are currently thought to be the most widespread fish species worldwide (Krumholz 1948; Lloyd and Tomasov 1985; Lloyd 1986). The widespread distribution of these livebearing (ovoviviparous) species, native to southern North America, has been mostly attributed to human assisted translocations aimed at controlling mosquito populations (Wilson 1960; Rivas 1963; Wooten *et al.* 1988; Courtenay and Meffe 1989; Lloyd 1990a). However, research undertaken over the last decades has demonstrated that *Gambusia* are relatively ineffective as mosquito control agents, and can instead have negative impacts on many aquatic species in non-native habitats (Lloyd 1990b; Garcia-Berthou 1999). Impacts include competition and direct predation on native fishes (Howe *et al.* 1997; Ivanstoff and Aarn 1999) and frogs (Hamer *et al.* 2002b; Kats and Ferrer 2003), as well as facilitating algal blooms following intense zooplankton predation (Hurlbert and Mulla 1981).

Now established on every continent except Antarctica (Lloyd and Tomasov 1985; Pyke 2005), the success of this small (up to 6 cm TL), non-descript fish can be attributed to a number of factors. In particular, *Gambusia* are hardy and able to survive in a wide range of environmental conditions (Pyke 2005). They have a relatively fast life cycle and exhibit a high degree of plasticity in life history characteristics (Meffe 1991; Haynes and Cashner 1995; Pyke 2005). In addition, they are generalist feeders able to exploit and adapt to changes in food resources in new environments (Arthington and Marshall 1999; Specziar 2004). Combined, these attributes are believed to contribute to the establishment of *Gambusia* throughout the world.

Gambusia holbrooki were first introduced in mainland Australia in the early 1900s

(Wilson 1960), and rapidly became established in all states except Tasmania (Arthington and Lloyd 1989; Clunie *et al.* 2002; Morgan *et al.* 2004). However, in 1992 some 50 mosquitofish were introduced into a private dam in northern Tasmania (Tasmanian Inland Fisheries Service [IFS hereafter], *unpublished data*). These fish were thought to have been subsequently eradicated from the area until late 2000, when a reproductively active population was found in the calm brackish waters of the Tamar Island Wetlands Reserve, in the upper reaches of the Tamar Estuary (Keane and Neira 2004).

As it was unclear how far *Gambusia holbrooki* had spread since their initial introduction, the broad aim of this chapter is to describe the ecology and distribution of *Gambusia* in the Tamar Estuary. Specifically, the extent and rate of spread of this introduced species is examined throughout the upper Tamar Estuary, northern Tasmania. Temporal patterns of abundance, diet, sex-structure and reproduction of an established *G. holbrooki* population are described. The likely impact of *Gambusia* on Tasmanian frog species and other native aquatic fauna is examined, and the future implications of range expansion of this pest fish throughout Tasmania are discussed.

2.2 Materials and methods

2.2.1 Study area

The Tamar Estuary is a 70km-long, partially-mixed, highly flushed system joined to the sea in Bass Strait by a narrow entrance channel, and fed by the North and South Esk rivers in the much narrower upstream reaches at Launceston (Foster *et al.* 1986); Fig. 2.1). The estuary is subjected to semi-diurnal tides, allowing for unrestricted fish movement

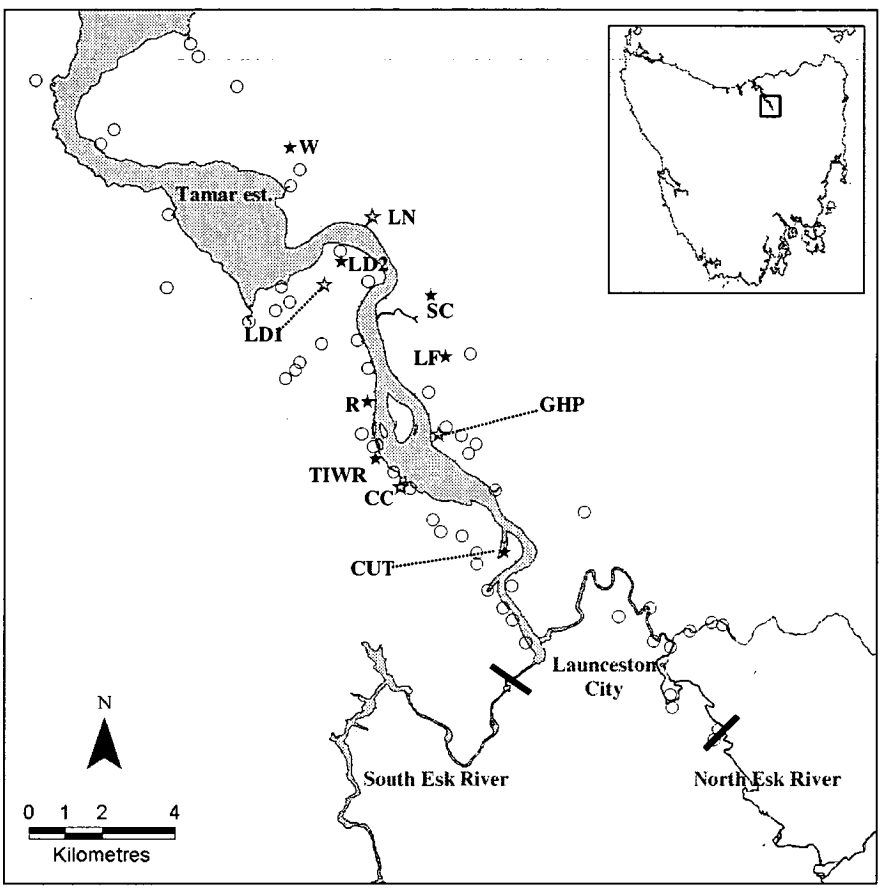

Figure 2.1. Distribution of *Gambusia holbrooki* in northern Tasmania (1992-2006). Solid stars represent sites currently occupied by *Gambusia*. Open stars represent *Gambusia* populations that have not persisted, and circles represent survey sites where *Gambusia* have never been encountered. Solid bars across the South and North Esk rivers indicate the extent of tidal inundation. Full names of study sites and descriptions are provided in Table 2.1.

Table 2.1. Habitat descriptions and density estimates (year of discovery in parentheses) for sites where *Gambusia holbrooki* was found between 2004 and 2006. (E) or (W) denotes eastern or western side of estuary respectively. Density estimates are based on direct observations only. Tick (✓) denotes year long tidal connectivity of the site with the main estuary channel and that macrophytes occur at the site. Cross (x) indicates no tidal connection with the estuary, except in flood conditions and that no macrophytes are present at the site. (*) indicates sites where *Gambusia* have not persisted.

Site code	Name/Location	Connection with estuary	Description	Macrophytes	*Gambusia* density
W	Windemere (E)	✓	large wetland with regular tidal inundation (lotic)	✓	High in isolated ponds (2006)
LN*	Lady Nelson Creek, Dilston (E)	✓	Rocky bottom tidal creek with high winter water flow (lotic)	x	Very Low (< 20 indiv.) (2004)
LD1*	Legana Dam 1 (W)	x	Small farm dam, (lentic)	✓	Eradicated in 2002 (1992)
LD2	Legana Dam 2 (W)	x	Small farm dam, (lentic)	✓	Very High (2002)
SC	Station Creek (E)	✓	Small, soft bottom tidal creek, some winter flow (lotic)	✓	Low (2005)
LF	Landfall (E)	x	Network artificial drainage channels, (lentic)	✓	High (2004)
R	Riverside Dam (W)	✓	Small farm dam, (lentic)	✓	High in vegetated margins (2004)
TIWR	Tamar Island Wetlands Reserve (W)	✓	large wetland with regular tidal inundation (lotic)	✓	High in vegetated margins (2001)
GHP*	Green Hillock Point (E)	✓	Open wetland (lotic)	x	Very Low (1 indiv.) (2004)
CC*	Cormiston Creek (W)	✓	Rocky bottom tidal creek with high winter water flow (lotic)	x	Very Low (< 20 indiv.) (2004)
CUT	The Cut (W)	✓	Artificial channel with regular tidal flow (lotic)	✓	Low, found in 1 isolated pond (2006)

throughout the system up to the Cataract Gorge on the South Esk, which forms a natural physical barrier to further fish dispersal. Approximately 50 *Gambusia* were first introduced in 1992 to a small farm dam in a rural area north of Launceston (LD1; Fig. 2.1), which subsequently spread into another nearby dam (LD2). Following several eradication attempts, the fish were thought to have been eliminated from the area until late 2000, when they were discovered in the Tamar Island Wetlands Reserve (TIWR), some 5 km south of the original dam (Fig. 2.1; see Table 2.1 for site codes and descriptions). Site LD2 in Legana (41°21' S; 147° 03'E) is a relatively small dam (1,048 m^2, 135m circumference) with slightly turbid water, and a muddy substrate covered in a dense mat of macrophytes and emergent reeds around the edge. While there is no permanent tidal connection with the Tamar Estuary (Table 2.1), it is possible for the dam to overflow at high water levels thereby allowing fish to enter the estuary (*pers. obs.*).

2.2.2 Sampling procedures

A total of 177 sites were sampled throughout the Tamar Estuary during the late summer of 2004-06 to determine the extent and rate of spread of *G. holbrooki*. The rationale for a late summer sampling was based on data from elsewhere in Australia indicating the species is most abundant at this time (Pen and Potter 1991; Pyke 2005). In all surveys, mosquitofish were captured by hand using 30cm diameter, 0.5mm mesh dip-nets. Occurrence data from all three years were combined and plotted on a single spatial distribution map of the Tamar Estuary using Arcview (GIS3.3 ESRI, 2002).

Mosquitofish and tadpoles were sampled monthly at LD2 during 2004-06 using a purpose-built, 0.5m^2 aluminum throw trap, which was cleared of fish and tadpoles using a

rigid framed tightly fitting net (Fig. 2.2). A stratified sampling design was employed on each sampling occasion, whereby the perimeter of the dam was divided into 10m randomly-selected transects to deploy the throw trap. At least 20 replicate trap throws were undertaken on each sampling month, and fish were euthanized using MS-222, fixed in 10% formalin, and later preserved in 70% ethanol. Temporal differences in *Gambusia* abundance at LD2 were tested using ANOVA; homogeneity of variance was determined by examining residual plots and data were $\log_{10}$ transformed when the assumption of equal variance was violated. All fish were retained for subsequent reproduction and dietary analyses. All tadpoles were counted, identified to species level and returned live to the place of capture.

The eradication of *Gambusia* from LD2 in June 2005 (methods described in Chapter 4) led to their temporary elimination from that site, allowing for a comparison of the tadpole community with and without *Gambusia.* Two-way ANOVA was performed to compare monthly abundance of tadpoles before and after mosquitofish eradication, while a one-way ANOVA with planned contrasts was used to compare corresponding months. Homogeneity of variance was determined by examining residual plots, and data were $\log_{10}$ $x+1$ transformed when this assumption was violated. All tadpole species as well as unidentified individuals (too small to identify to species) were pooled for the analyses.

Water temperatures at LD2 were recorded every 30 min using a temperature data logger, and averaged per month; values reported herewith correspond to maximum averages. Regression analyses were used to predict maximum water temperature from maximum daily air temperatures supplied by the Bureau of Meteorology in the absence of field data. Mosquitofish abundances and mean monthly temperatures were compared using the non-parametric Spearman's rank correlation analysis. All statistical analyses were

carried out using the software package SPSS (ver. 14.0 for windows, SPSS Inc.).

Figure 2.2. (a) Throw trap (0.5 m^2) for sampling *Gambusia* and tadpoles, and (b) net used to clear the trap.

2.2.3 Laboratory procedures

The standard length (SL) and weight (g) of each mosquitofish was obtained to the nearest 0.01 mm and 0.001 g, respectively, and recorded by sex. Individuals were classified as "male" if they possessed a gonopodium (either fully or partially developed), as "female" if they were larger than the smallest male and possessed no evidence of a gonopodium, and as "juvenile" if they did not fit either of the above categories. Ovaries of "pregnant" females were weighed to the nearest 0.001g, and ovaries and embryos (in all developmental stages) staged according to (Keane and Neira 2004). The ovariosomatic index (OSI = [ovary wt/body wt] $\times$ 100) was calculated for all pregnant females (Keane and Neira 2004). Data were $\log_{10}$ transformed, and tested for significant differences in monthly OSI using one-way ANOVA.

Dietary analyses were undertaken to identify potential impacts of *Gambusia* on native aquatic species. The stomach contents of all mosquitofish >15 mm SL from monthly samples were examined, identified and allocated to one of 18 prey categories using the points method (Hynes 1950), which provides a relative contribution of each prey item to the volume of the stomach content of each fish. Data from 30 randomly selected fish from two size (mm SL) classes (medium >15-≤25; large >25) and all seasons were analyzed using PERMANOVA (Anderson 2005) to examine diet differences among size classes. Monthly data were pooled in order to examine seasonal variation in diet. Average seasonal diversity of prey items was calculated as the average of the monthly total number of prey items in the diet for both medium and large fish in each month of each season (n=6).

2.3 Results

2.3.1 Distribution and abundance

The late summer 2004 surveys along the Tamar Estuary revealed five new populations of *Gambusia holbrooki* (LN, LF, GHP, R and CC), three of which were located on the eastern shore of the estuary (Figs 2.1, 2.3). However, the 2005 surveys failed to locate any populations at LN, GHP or CC, while in 2006 two new populations (W and CUT) were discovered to the north and south of the previous range, respectively. Mosquitofish abundance at LD2 fluctuated significantly throughout the study period ($F = 38.58$, $df = 11, 211$, $P < 0.001$), with mean abundances during the summer 2004-05 reaching ~60 fish 0.5 m^{-2} (Fig. 2.4). There was a significant positive correlation ($R = 0.636$, $P< 0.05$) between mean water temperature and *Gambusia* abundance at LD2 over the sampling period (Fig. 2.4).

2.3.2 Reproductive status

Synchronous births of mosquitofish around mid November were observed during 2004-06 at LD2, R and TIWR. Juveniles were absent from LD2 in October, whereas all females carried developing embryos by November (Fig. 2.5). Brood sizes ranged from one in June 2004 to 124 in December 2004. The ovariosomatic index (OSI) changed significantly during the study period ($F = 28.13$, $df = 8, 132$, $P < 0.001$), with the highest average values (22.6 - 21.4) obtained at the beginning of the reproductive season in November – January 2004/05 (Fig. 2.5). The proportion of juveniles in the population

peaked in December (83.9%), with a smaller peak in February (66.1%) due to a second cohort (Fig. 2.5). The proportion of males in the LD2 population was consistently low except in March 2005 (Fig. 2.5).

2.3.3 Diet

Mosquitofish diet at LD2 varied significantly with season and length size class of fish ($P<0.05$). Winter diet of medium (>15 and ≤25 mm SL) and large (>25 mm SL) fish did not differ significantly ($P>0.05$), while there was a significant difference in autumn ($P<0.05$), spring and summer ($P<0.001$). The proportion of fish with empty stomachs was highest in June and July (Table 2.2). Diversity of prey items was highest in November (16) and lowest in August (4), with crustaceans (predominantly micro-crustaceans such as ostracods, copepods and isopods) comprising the preferred prey of both size classes of fish throughout the year except for large fish in summer which fed mainly on molluscs (Table 2.2). Mosquitoes occurred in the diet in small numbers in autumn and spring, while amphibians (including frog eggs, tadpoles and adult frogs) made up a very minor proportion of the spring diet, e.g. 2.6% in November 2004 (Table 2.2).

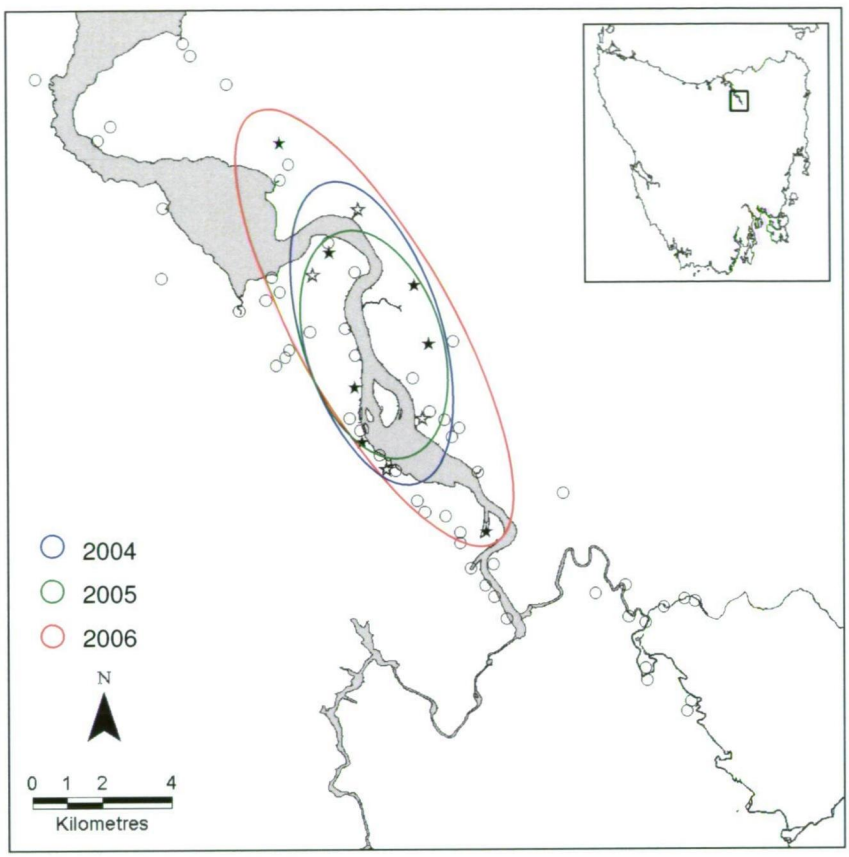

Figure 2.3. Spread of *Gambusia* in the Tamar Estuary region from 2004 to 2006. Hollow circles (o) indicate surveyed sites where *Gambusia* were not detected. Stars (★) represent sites where *Gambusia* were present and hollow stars (☆) indicate *Gambusia* populations that did not persist to the next survey year.

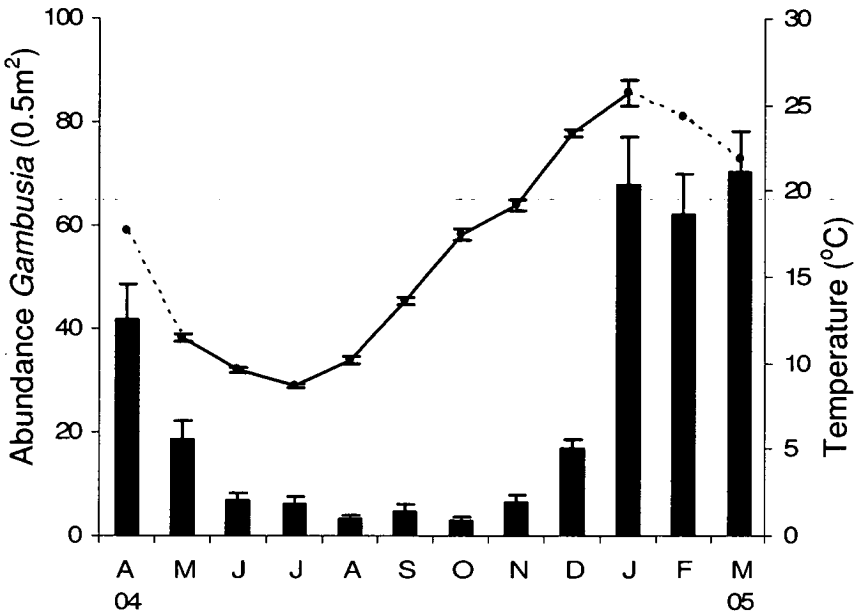

Figure 2.4. Temporal changes in the monthly mean (± SE) abundance of *Gambusia holbrooki* (bars) and water temperature (°C ± SE) (lines) from Legana Dam 2 in Tasmania. Solid and dotted lines represent actual and predicted temperature respectively [Predicted monthly average water temp = (average monthly air temp * 1.472) – 9.965; regression $R^2 = 0.984$]

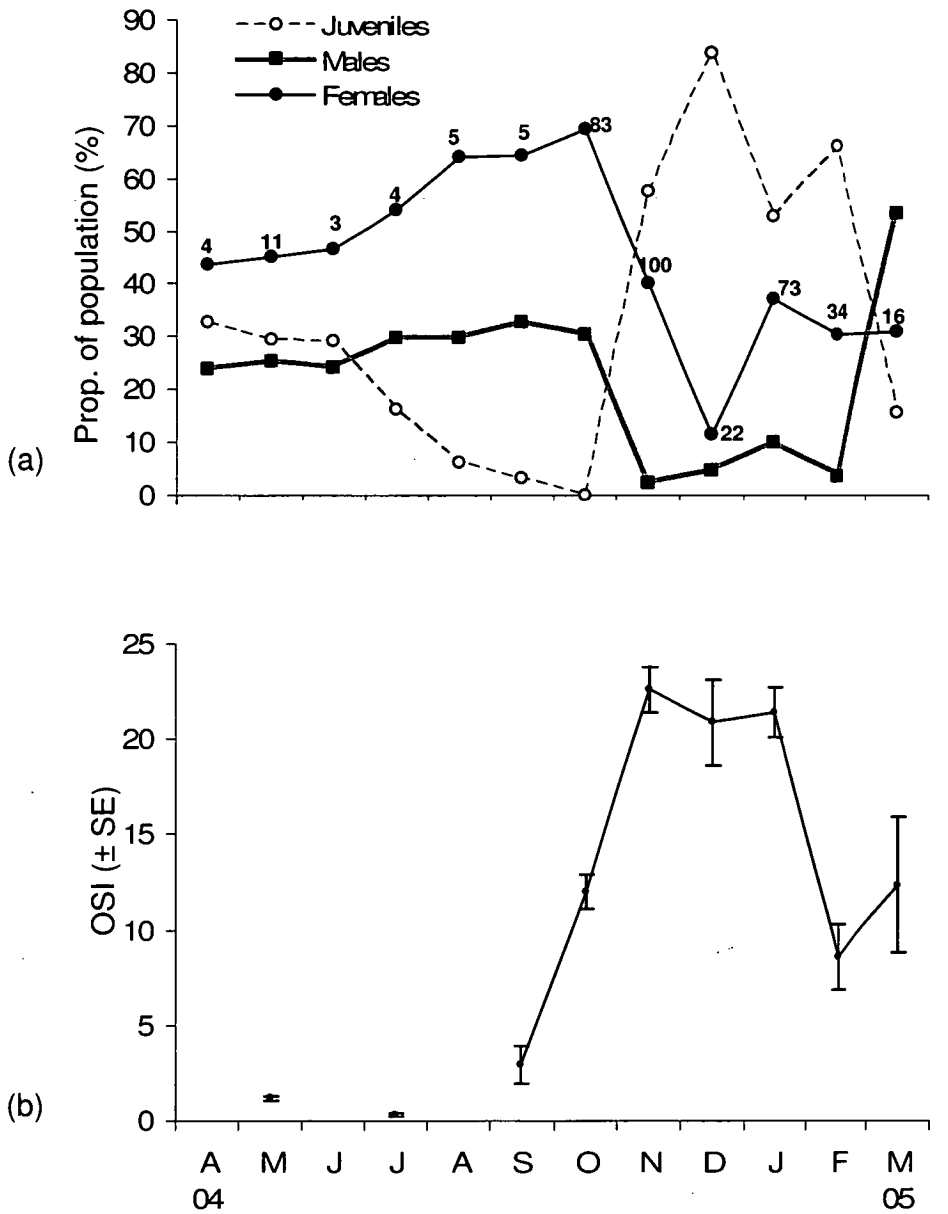

Figure 2.5. Temporal changes in the sex structure of *Gambusia* populations (a) and ovariosomatic index OSI (b) at LD2 in 2004/05. Values indicate the proportion (%) of sampled females with developing embryos.

Table 2.2. Seasonal diet of *Gambusia holbrooki* at Legana Dam 2 in northern Tasmania. Monthly data are pooled into seasons. Values in bold indicate the most utilized prey type in each season. Average diversity of prey items is the seasonal average of total number of prey categories in the diet per month across all size classes of fish.

	Autumn		**Winter**		**Spring**		**Summer**	
	Medium n =198	**Large** n =30	**Medium** n =212	**Large** n =37	**Medium** n =110	**Large** n =92	**Medium** n =78	**Large** n =30
Diet (% contribution)								
Unidentified	1.5	2.2	0.5	-	0.1	2.0	6.0	2.3
Diptera adult	25.3	22.4	0.4	11.0	7.1	15.9	6.5	9.9
Diptera larvae	4.9	-	-	-	4.4	5.6	1.7	1.2
Molluscs	10.8	6.3	8.1	13.7	5.8	20.0	20.0	**45.7**
Ants	-	6.7	-	-	-	0.3	-	0.3
Mites	0.1	0.4	1.2	4.0	1.0	1.3	-	0.3
Detritus	2.4	3.1	-	-	0.5	2.2	1.3	7.4
Crustacea	**44.7**	**30.2**	**89.1**	**66.4**	**70.7**	**22.3**	**37.0**	10.2
Chironomids	-	-	-	1.0	1.4	0.5	4.7	0.3
Mosquitoes	0.6	0.7	-	-	0.4	0.2	-	-
Hemiptera	1.9	8.3	-	1.8	1.9	2.2	6.4	1.5
Coleoptera larvae	1.2	3.6	0.2	0.2	-	1.3	8.0	3.7
Coleoptera adult	0.3	5.4	0.2	0.7	0.5	1.1	0.8	1.2
Collembollans	2.3	5.3	0.2	1.2	0.8	0.1	-	-
Odonata & Caddis	0.6	-	-	-	1.5	19.6	-	7.4
Gambusia	2.8	5.4	-	-	-	1.8	3.4	5.0
Amphibians	-	-	-	-	-	1.5	-	-
Other	0.7	-	-	-	3.8	2.1	4.1	3.4
Average diversity of prey items (SE)	9.3 (1.05)		5.6 (0.91)		11.3 (1.28)		7.5 (1.43)	
Prop. of pop. with empty Stomachs (%)	1.75 %		8.03 %		< 1 %		< 1 %	

2.3.4 Impact on tadpoles

A total of 131 tadpoles from three species were caught at LD2 during the breeding season from September 2004 to April 2005, namely *Crinia signifera* (common froglet), *Limnodynastes dumerilii insularis* (pobblebonk) and *Litoria ewingii* (brown tree frog). Mean tadpole abundance (numbers/0.5 m^2, all species pooled) declined from 1.95 in December 2004 to 0.1 in April 2005 (Fig. 2.6). Obvious damage to the tail region (Fig. 2.7) was evident in many tadpoles caught in November 2004 and February 2005 (the end of the breeding season), when mosquitofish abundance was highest. By contrast, there was a significant increase in tadpole abundance at LD2 in the months following the mosquitofish eradication from the dam ($F = 19.52$, $df = 4, 190$, $P < 0.001$), with 1,050 tadpoles from four species caught at LD2 between September 2005 and March 2006. Species captured during the latter season included the three recorded during the 2004-05 season as well as *Lymnodynastes tasmaniensis* (spotted marsh frog). Mean abundances ranged from 0.3 to 30 tadpoles per 0.5 m^{-2} in October 2005 and March 2006, respectively (Fig. 2.6). Tadpole abundance was significantly higher in all months when *Gambusia* were absent ($F = 38.61$, $df = 9, 190$, $P < 0.001$) except when comparing January 2005 to January 2006 ($P = 0.082$). Unlike tadpoles caught during the 2004-05 season, those caught during 2005-06 showed no damage to tails.

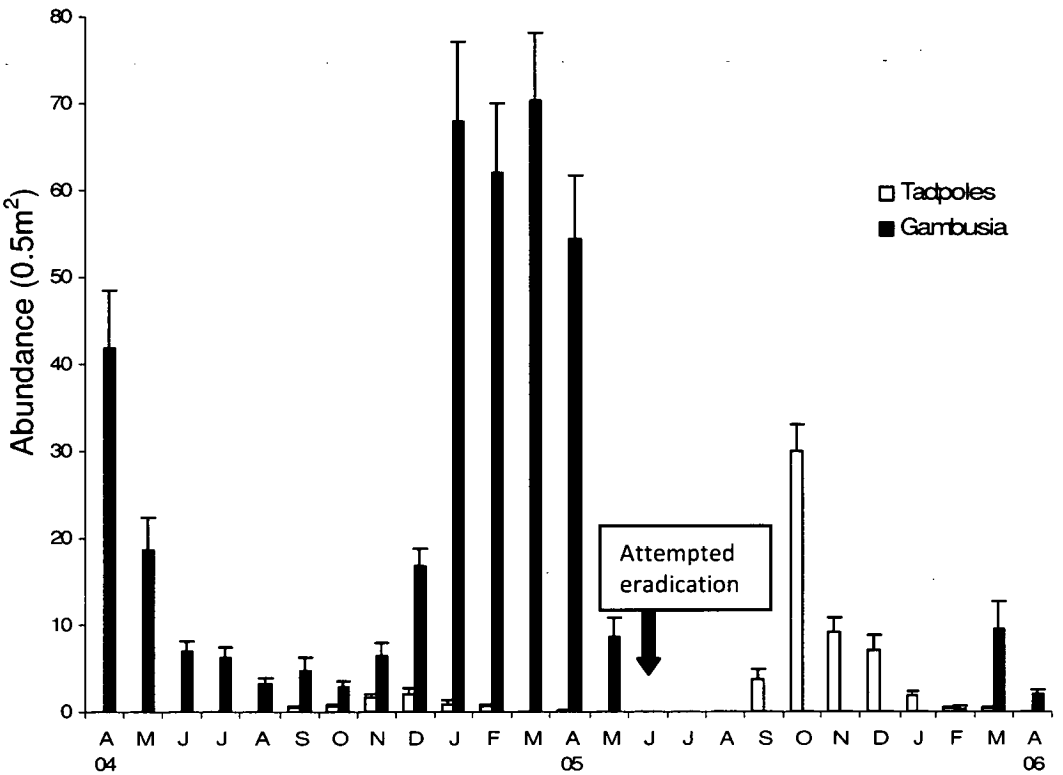

Figure 2.6. Abundance of *Gambusia* and tadpoles (all species) at LD2 in northern Tasmania. Error bars are standard errors.

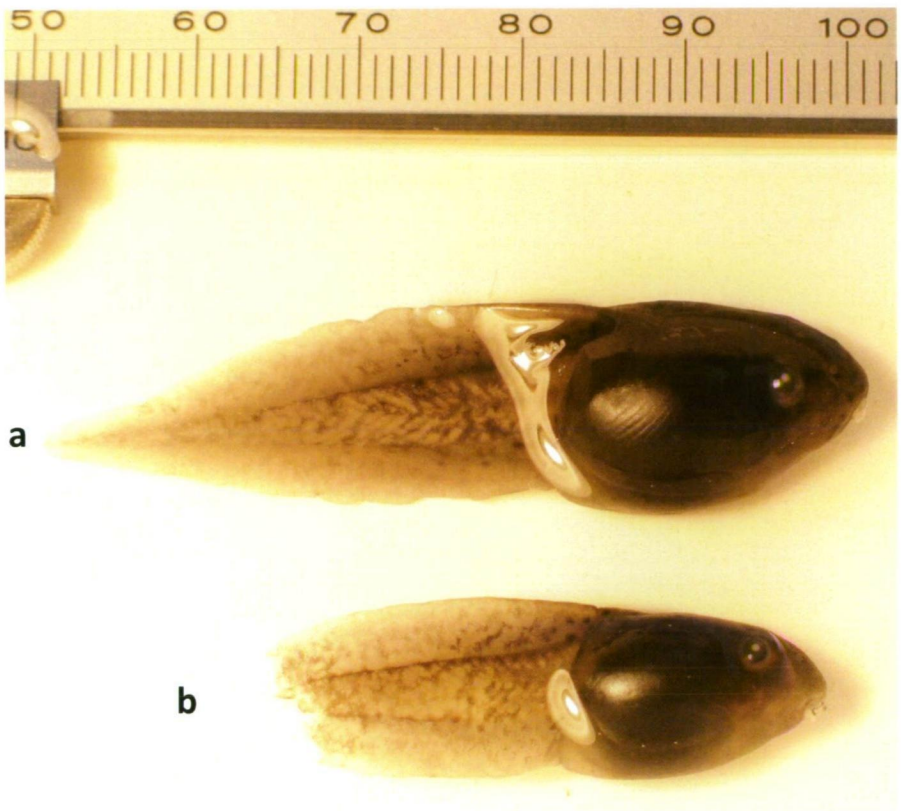

Figure 2.7. Comparison of **(a)** a normal *Limnodynastes dumerilii insularis* tadpole and **(b)** and an individual of the same species with tail damage observed at LD2.

2.4 Discussion

2.4.1 Distribution

This study identified at least seven, reproductively active populations of *Gambusia* in the Tamar Estuary during the study period in 2004-06. Five of these populations found during late summer 2004 represented a substantial increase in the northern range of this species from the initial introduction sites at LD2 and TIWR. Considering all established populations, the current mosquitofish distribution in the Tamar covers approximately 18 km of habitat bordering the upper estuary, this translates to a rate of spread of less than 2 km per year over the 14-years since their introduction in 1992. It is difficult to compare the recent Tasmanian introduction with other *Gambusia* introductions because the vast majority occurred many decades ago and were poorly documented. However, when compared to other invasive species in a range of habitat types, the rate of spread described for *Gambusia* in the Tamar Estuary is modest. For example, (Grosholz 1996) compares range expansion in terrestrial vs. marine environments and showed that introduced species in terrestrial systems (mean 89 km yr^{-1}) invaded at a slightly higher rate than those in marine systems (mean = 50.7 km yr^{-1}). Both figures are considerably higher than the rate reported in this study. In the Baltic Sea, estimates of the secondary rate of spread of non-indigenous species varied from 30-480 km yr^{-1} across a range of taxonomic groups (Leppakoski and Olenin 2000). Although not an estimate of linear spread, (Pusey *et al.* 2006) found that the sleepy cod *Oxyeleotris lineolatus* took less than a decade to invade the entire Burdekin River tributary system (130 000 km^2), reporting a lag phase lasting a decade during which the fish were restricted to the point of introduction.

The slow rate of spread of *Gambusia* in the Tamar Estuary is not surprising given that the invasion is quite recent. A lag phase in population abundance and dispersal tends to occur after initial introduction, characterized by a period of little or no active range expansion by the invader in the non-indigenous habitat (Crooks and Soule 1999; Allendorf and Lundquist 2003). The biological causes of lag phases are relatively poorly understood, but can be caused by the nature of population growth and range expansion. They can be caused when an alien species is introduced into an environment with unfavourable ecological conditions. The alien species may remain in a lag phase until there is a change in the environmental conditions which favours the alien species over the native species. There may also be a genetic basis for a lag phase in alien species where time is required for the evolution of adaptations to the new environment (Crooks and Soule 1999). Environmental and/or economic impacts are generally not seen during a lag phase of invasion (Sakai *et al.* 2001). Slow initial rates of spread are often a reflection of a lag phase that can lead to the underestimation of future potential spread of the species in question (Allendorf and Lundquist 2003). Furthermore, the rate of spread of a new invader is not an indicator of future potential impact (Ricciardi and Cohen 2007). The results of this study suggests that the *Gambusia* invasion of the Tamar Estuary is in a lag phase as negative environmental or economic impacts appear minimal, spread is slow, and although some populations have become established others have been unable to persist over time. It is likely that the lag phase has been caused by an environmental interaction, and we know that although clearly capable of surviving in estuarine conditions, colonization of estuaries by *Gambusia* is not widespread (Arthington and Lloyd 1989).

Mosquitofish generally prefer shallow, still or slow moving water, with dense

aquatic vegetation (macrophytes) that provides shelter from predators (Casterlin and Reynolds 1977; Ward *et al.* 2003). Tamar Estuary *Gambusia* populations are no exception, as populations have become established in predominantly lentic habitats, in the presence of macrophytes (*see* Table 2.1). Some populations surveyed here are established in lotic habitats, however water flow at these sites is minimal. It is likely that at the sites where *Gambusia* have not persisted (LN, CC and GHP) the habitat conditions were unfavourable (e.g. high water flows and no macrophytes). At these sites *Gambusia* would be flushed out during high flows, and in low flow conditions are unable to find refuge from predators (Meffe 1984; Ward *et al.* 2003; Chapman and Warburton 2006).

The majority of sites colonized by *Gambusia* in the Tamar region are tidally connected to the estuary allowing unrestricted movement of fish among them (Table 2.1). LD1, LD2 and LF are impoundments where there is a physical barrier to fish dispersal (Table 2.1). However, periodic flood events allow for fish movement both into and out of these sites. Therefore, it appears likely that the spread of *Gambusia* in the Tamar Estuary is entirely due to the natural dispersal of fish and not associated with human translocation (with the exception of the initial introduction). Although *Gambusia* do not generally prefer or thrive in estuarine conditions (Arthington and Lloyd 1989), the gradual spread to suitable habitat along the estuary margins indicates that *Gambusia* will continue their gradual range extension to all tidally connected suitable habitat along the Tamar and North Esk rivers. Natural range expansion outside of the Tamar Estuary is impossible due to the lack of connectivity between the estuary and other water bodies in the region. However, colonization of Tasmania's inland waterways by *Gambusia* could occur via human assisted translocation (by deliberate or unintentional pathways; see Chapter 5). Hence, *Gambusia* poses a significant threat to Tasmania's native aquatic fauna in areas,

such as the central highland lakes, where habitat characteristics are particularly suitable to the species.

2.4.2 Population dynamics

Gambusia often account for a large proportion of the total fish captured or observed at a particular location (Arthington and Milton 1983; Morton *et al.* 1988). *Gambusia* abundances are also generally higher in introduced populations compared to native range populations that are thought to be regulated by co-evolved predators and parasites (Meffe and Snelson 1989; Lloyd 1990a). Given the vast amount of literature on *Gambusia*, there are surprisingly few quantitative estimates of *Gambusia* abundance. This is most likely due to the difficulties of finding a sampling method suited to the wide range of habitats where *Gambusia* can be found. Some reported *Gambusia* abundance estimates are 2-10 individuals m^{-2}, 0-2.7 per linear m of stream, <10-320 per 100 m^{-2} of stream, 80-430 m^{-3}, and 49-71 m^{-3} (Deacon and Bradley 1972; Pen and Potter 1991; Zulian *et al.* 1993; Schaefer *et al.* 1994; Goldingay and Lewis 1999). Although it is unwise to compare abundance estimates among studies where a range of sampling techniques and methodologies have been utilized, *Gambusia* abundances observed in this study are in a similar range to those reported elsewhere.

Many studies have observed a reduction in *Gambusia* population abundance over the cooler months of the year (Hughes 1985; Pen and Potter 1991; Zulian *et al.* 1993). Prior to this study, it was unclear if lower observable population abundances were due to reduced population sizes or sheltering behavior that may make the fish less observable (Pyke 2005). The sampling method (throw trap) used in this study ensured that all fish

present in the water column and the top layers of mud were captured in the sample. Therefore, lower winter population densities recorded in our study are a more accurate indication of reduced population size. The observed reduction in population size is probably a result of senescence of the majority of individuals in the population following reproduction (Haynes 1993; Vargas and de Sostoa 1996). A small proportion of the population is then able to survive over-winter and initiate population growth in the subsequent breeding season (Haynes 1993; Vargas and de Sostoa 1996).

Tasmanian *Gambusia* populations exhibited synchronous birth from 2004-2006, with reproductive output (OSI) greatest in early summer. Water temperature, and to a lesser extent photoperiod, control reproduction in *Gambusia* (Koya and Kamiya 2000; Pyke 2005). Reproduction by female *Gambusia* ceases when water temperatures are <16°C (Medlen 1951), and in this study virtually no females with developing embryos were observed when the average monthly water temperature was <16°C (May–Sep). Synchronous birth events in *Gambusia* populations have been observed in the USA and elsewhere (Krumholz 1948; Hughes 1985; Haynes and Cashner 1995). These events can be a result of high levels of over-winter mortality leading to a population with females all of roughly the same size. This happens in more northern USA populations where the winters are harsher and the environment less suitable to *Gambusia* (Haynes and Cashner 1995). *Gambusia* do not generally occur further north than 38 °N in their native north American range (Krumholz 1948). Therefore Tasmania (latitude 41°S) presents environmental conditions at the limits of *Gambusia*'s range. However, they can survive and over-winter at higher latitudes (Smith 1960). Hence, the relatively cold Tasmanian winters appear to be the main factor influencing seasonal abundance and reproduction.

2.4.3 Diet and impacts on Tasmanian native species

The diet of Tasmanian *Gambusia* found during this study is similar to that reported in many other studies. In particular, the diet is broad, variable with season, consists mainly of zooplankton and other invertebrates, and only a small portion is made up of mosquitoes (Pen and Potter 1991; Mansfield and McArdle 1998; Garcia-Berthou 1999; Specziar 2004). A broad diet and the ability to exploit seasonally variable food sources has undoubtedly helped *Gambusia* to establish populations in newly invaded habitats. This attribute, combined with other factors, such as wide physiological tolerance (Pyke 2005), plasticity in life history characteristics (Pyke 2005) and the ability to establish in areas subject to human disturbance (Arthington and Milton 1983), ensure that *Gambusia* are able to fully exploit the available resources in non-native habitats. This ultimately leads to negative impacts on native species and habitats where they have been introduced.

Examples of negative impacts in non-native environments attributed to *Gambusia* are numerous and have been well documented (Courteney and Meffe 1989). This study examined actual and potential impact of *Gambusia* on Tasmanian amphibians (namely frog eggs and/or tadpoles) because the two groups have similar habitat requirements and coexisted at a number of the study sites. There are many examples in the literature of *Gambusia* predation on amphibians, or where they have been implicated in the decline of adult frog populations (Webb and Joss 1997; Goodsell and Kats 1999; Lawler *et al.* 1999; Komak and Crossland 2000; Hamer *et al.* 2002b; Kats and Ferrer 2003). Interestingly, in this study there was very little evidence of direct predation (1.5% of the diet of large fish in spring) on any of the four frog species that coexisted with *Gambusia* at LD2. However,

there was a significant increase in the size of the tadpole community when *Gambusia* were absent, suggesting that *Gambusia* may play a major role in limiting tadpole populations. *Gambusia* can negatively impact frog populations by directly competing with tadpoles for food and other resources, and by interference competition in the form of fin-nipping on the tail of tadpoles (Lawler *et al.* 1999; Kats and Ferrer 2003). This type of aggressive behaviour by *Gambusia* can lead to reduced survival, recruitment and growth in tadpoles, delayed metamorphosis and lower weight of immature frogs (Lawler *et al.* 1999; Kats and Ferrer 2003). Evidence of fin-nipping in the form of damage to the tails of tadpoles was observed in the summer of 2004/05, when *Gambusia* densities were highest at LD2. This evidence, together with the increase in the tadpole community when *Gambusia* were absent, supports the hypothesis that *Gambusia* were actively competing (either for food, space or other resources) with the tadpole species at the study site.

Some frog species are able to coexist with *Gambusia* if there is adequate aquatic vegetation to provide cover, or if their mode of reproduction does not coincide with peak *Gambusia* densities (Ghate and Padhye 1988; Pyke and White 1996; Hamer *et al.* 2002a). In this study the frogs began to breed when *Gambusia* densities were still low (September in both years), avoiding a major overlap with highest summer densities of *Gambusia* and therefore promoting coexistence.

Other Tasmanian frog species (not encountered at LD2) such as *Litoria burrowsae* (endemic Tasmanian tree frog) and the green and gold frog, *Litoria raniformis* are potentially at risk from *Gambusia* due to their similar habitat requirements. Both species prefer to breed in lentic habitats and will utilize artificial water bodies such as roadside pools and dams to breed (Pyke 2002; Littlejohn 2003). *Litoria raniformis* was listed as an endangered species in 2002 and has shown a dramatic reduction in range over the last

several decades (Mahony 1999). This frog has been observed breeding at two sites (TIWR and R) that *Gambusia* currently inhabits in the Tamar Estuary and its breeding season coincides with *Gambusia* (*pers. obs.*). Stresses associated with the introduction of a new predator or competitor can be devastating to populations that are already declining (Kats and Ferrer 2003). For these reasons, establishment of *Gambusia* populations at *L. raniformis* breeding sites may lead to severe reductions in reproductive success or even local extinction of the frog species. Although the range of *L. burrowsae* does not currently overlap with *Gambusia* (Littlejohn 2003), it is vulnerable to negative impacts in the event of further range expansion by *Gambusia* because, like *L. raniformis*, its reproductive activity and habitat requirements are similar.

This study has shown that while *G. holbrooki* does not spread rapidly via natural dispersal it is capable of negatively affecting native frog species in lentic habitats where it can reach high population abundances. *Gambusia* cannot establish in all new habitats that it encounters. While *Gambusia* remains restricted to the Tamar Estuary the potential for negative environmental impacts is relatively low because habitat conditions are not always optimal and there is currently minimal overlap with potentially at-risk species. However, as the dispersal of these fish is so often linked to deliberate and/or accidental human assisted translocation, the possibility of its further spread in Tasmania seems highly likely. In the event that *Gambusia* does gain access to Tasmania's inland waterways significant negative impacts on native aquatic fauna should be expected.

2.5 References

Allendorf F. W. and Lundquist L. L. (2003) Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Anderson M. J. (2005) 'PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. (Ver. (Department of Statistics, University of Auckland: New Zealand).

Arthington A. H. and Lloyd L. N. (1989) Introduced Poeciliids in Australia and New Zealand. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 333-348. (Prentice Hall: New Jersey).

Arthington A. H. and Marshall C. J. (1999) Diet of the exotic mosquitofish *Gambusia holbrooki* in an Australian lake and potential for competition with indigenous fish species. *Asian fisheries Science* **12**, 1-16.

Arthington A. H. and Milton D. A. (1983) Effects of urban development and habitat alterations on the distribution and abundance of native and exotic freshwater fish in the Brisbane region, Queensland. *Australian Journal of Ecology* **8**, 87-101.

Casterlin M. E. and Reynolds W. W. (1977) Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia* **55**, 125-127.

Chapman P. and Warburton K. (2006) Postflood movements and population connectivity in gambusia (*Gambusia holbrooki*). *Ecology of Freshwater Fish* **15**, 357-365.

Clunie P., Stuart I., *et al.* (2002) 'A risk assessment of the impacts of pest species in the riverine environment in the Murray-Darling Basin.' Department of Natural Resources and Environment, Arthur Rylah Institute for environmental research, Project R2006, Heidelberg, Victoria.

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Crooks J. A. and Soule M. E. (1999) Lag times in population explosions of invasive species: causes and implications. In 'Invasive species and biodiversity management'. (Eds OT Sandlund, PJ Schei and A Viken) pp. 103-126. (Kluwer Academic Publishers).

Deacon J. E. and Bradley W. G. (1972) Ecological distribution of fishes of Moapa (Muddy) River in Clark County, Nevada. *Transactions of the American Fisheries Society* **101**, 408-419.

Foster D. N., Nittim R. and Walker J. (1986) 'Tamar River siltation study.' University of New South Wales, 85/07, Manly Vale.

Garcia-Berthou E. (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* **55**, 135-147.

Ghate H. V. and Padhye A. D. (1988) Predation of *Microhyla* tadpoles by *Gambusia*. *Journal of the Bombay Natural History Society* **85**, 200-201.

Goldingay R. and Lewis B. (1999) Development of a conservation strategy for the green and golden bell frog in the Illawara region of NSW. *The Australian Zoologist* **31**, 376-387.

Goodsell J. A. and Kats L. B. (1999) Effect of Introduced Mosquitofish on Pacific Treefrogs and the Role of Alternative Prey. *Conservation Biology* **13**, 921-924.

Grosholz E. D. (1996) Contrasting rates of spread for introduced species in terrestrial and marine systems. *Ecology* **77**, 1680-1686.

Hamer A. J., Lane S. J. and Mahony M. J. (2002a) Management of freshwater wetlands for the endangered green and golden bell frog (*Litoria aurea*): roles of habitat determinants and space. *Biological Conservation* **106**, 413-424.

Hamer A. J., Lane S. J. and Mahony M. J. (2002b) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* **132**, 445-452.

Haynes J. L. (1993) Annual reestablishment of mosquitofish populations in Nebraska. *Copeia* **1993**, 232-235.

Haynes J. L. and Cashner R. C. (1995) Life history and population dynamics of the western mosquitofish: A comparison of natural and introduced populations. *Journal of fish biology* **46**, 1025-1041.

Howe E., Howe C., Lim R. and Burchett M. (1997) Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine & Freshwater Research* **48**, 425-434.

Hughes A. L. (1985) Seasonal changes in fecundity and size at first reproduction in an Indiana population of the mosquitofish *Gambusia affinis*. *American Midland Naturalist* **114**, 30-36.

Hurlbert S. H. and Mulla M. S. (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* **83**, 125-151.

Hynes H. B. N. (1950) The food of sticklebacks with a review of the methods used in studies of the food of fishes. *Journal of Animal Ecology* **19**, 36-58.

Ivanstoff W. and Aarn (1999) Detection of predation on Australian native fishes by *Gambusia holbrooki*. *Marine and Freshwater Research* **50**, 467-468.

Kats L. B. and Ferrer R. P. (2003) Alien predators and amphibian decline: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9**, 99-110.

Keane J. P. and Neira F. J. (2004) First record of mosquitofish, *Gambusia holbrooki*, in Tasmania: stock structure and reproductive biology. *New Zealand Journal of Marine and Freshwater Research* **38**, 857-868.

Komak S. and Crossland R. (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* **27**, 185-189.

Koya Y. and Kamiya E. (2000) Environmental Regulation of Annual Reproductive Cycle in the Mosquitofish, *Gambusia affinis*. *Journal of Experimental Zoology* **286**, 204-211.

Krumholz L. A. (1948) Reproduction in the western mosquitofish *Gambusia affinis affinis* (Baird and Girard) and its use in mosquito control. *Ecological Monographs* **18**, 1-43.

Lawler S. P., Dritz D., Strange T. and Holyoak M. (1999) Effects of Introduced Mosquitofish and Bullfrogs on the Threatened California Red-Legged Frog. *Conservation Biology* **13**, 613-622.

Leppakoski E. and Olenin S. (2000) Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biological Invasions* **2**, 151-163.

Littlejohn M. (2003) 'Frogs of Tasmania.' Fauna of Tasmania Handbook no. 6. 80 pgs. (University of Tasmania: Hobart).

Lloyd L. (1990a) Ecological interactions of *Gambusia holbrooki* with Australian native fish. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 94-97. (Austalian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. (1990b) Native fishes as alternative to the exotic fish, *Gambusia*, for insect control. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 115-122. (Australian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. N. (1986) An alternative to insect control by "mosquitofish", *Gambusia affinis*. In '4th Australian arbovirus symposium'. Brisbane. (Eds TD George, BH Kay and J Blok). (Arbovirus research in Australia).

Lloyd L. N. and Tomasov J. F. (1985) Taxonomic status of the mosquitofish, *Gambusia affinis* (Poeciliidae), in Australia. *Aust.J.Mar.Freshwat.Res.* **36**, 447-451.

Mahony M. J. (1999) Review of the declines and disappearances within the bell frog species group (*Litoria aurea* species group) in Australia. In 'Declines and Disappearances of Australian Frogs'. (Ed. A Campbell) pp. 81-93. (Environment

Australia: Canberra).

Mansfield S. and M^{c}Ardle B. H. (1998) Dietary composition of *Gambusia affinis* (Family Poeciliidae) populations in the northern Waikato region of New Zealand. *New Zealand Journal of Marine and Freshwater Research* **32**, 375-383.

Medlen A. B. (1951) Preliminary observations on the effects of temperature and light upon reproduction in *Gambusia affinis. Copeia* **1951**, 148-152.

Meffe G. K. (1984) Effects of abiotic disturbance in coexistence of of predator-prey fish species. *Ecology* **65**, 1525-1534.

Meffe G. K. (1991) Life history changes in eastern mosquitofish (*Gambusia holbrooki*) induced by thermal elevation. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 60-66.

Meffe G. K. and Snelson F. F. J. (1989) An ecological overview of the Poeciliid fishes. In 'Ecology and Evolution of livebearing fishes'. (Eds GK Meffe and FFJ Snelson) pp. 13-31. (Prentice Hall: New Jersey).

Morgan D. L., Gill H. S., Maddern M. K. and Beatty S. J. (2004) Distribution and impacts of introduced freshwater fishes in Western Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 511-523.

Morton R. M., Beumer J. P. and Pollock B. R. (1988) Fishes of a subtropical Australian saltmarsh and their predation upon mosquitoes. *Environmental Biology of Fishes* **21**, 185-194.

Pen L. J. and Potter I. C. (1991) Reproduction, growth and diet of *Gambusia holbrooki* (Girard) in a temperate Australian river. *Aquatic Conservation: Marine and Freshwater Ecosystems* **1**, 159-172.

Pusey B., Burrows D., Arthington A. and Kennard M. (2006) Translocation and spread of piscivorous fish in the Burdekin River, north-eastern Australia. *Biological Invasions* **8**, 965-977.

Pyke G. H. (2002) A review of the biology of the Southern Bell Frog *Litoria raniformis* (Anura:Hylidae). *Australian Zoologist* **32**, 32-48.

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki. Reviews in Fish Biology and Fisheries* **15**, 339-365.

Pyke G. H. and White A. W. (1996) Habitat requirements of the Green and Golden Bell Frog *Litoria aurea* (Anura: Hylidae). *The Australian Zoologist* **30**, 224-232.

Ricciardi A. and Cohen J. (2007) The invasiveness of an introduced species does not predict its impact. *Biological Invasions* **9**, 309-315.

Sakai A. K., Allendorf F. W., *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**, 305-332.

Schaefer J. F., Heulet S. T. and Farrell T. M. (1994) Interactions between two poeciliid fishes (*G. holbrooki* and *Heterandria formosa*) and their prey in a Florida marsh. *Copeia* **1994**, 516-520.

Smith D. L. (1960) The ability of the topminnow, *Gambusia affinis* (Baird and Girard) to reproduce and overwinter in an outdoor pond at Winninpeg, Manitoba, Canada. *Mosquito News*, 55-56.

Specziar A. (2004) Life history patterns and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of Lake Heviz, Hungary. *Hydrobiologia* **522**, 249-260.

Vargas M. J. and de Sostoa A. (1996) Life history of *Gambusia holbrooki* (Pisces, Poeciliidae) in the Ebro delta (NE Iberian peninsula). *Hydrobiologia* **341**, 215-224.

Ward D. L., Schultz A. A. and Matson P. G. (2003) Differences in swimming ability and behavior in response to high water velocities among native and nonnative fishes. *Environmental Biology of Fishes* **68**, 87-92.

Webb C. and Joss J. (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist* **30**, 316-326.

Zulian E., Bisazza A. and Marin G. (1993) Determinants of size in male eastern mosquitofish (*Gambusia holbrooki*): inheritance and plasticity of a sexual selected character. *Bollettino di Zoologia* **60**, 317-322.

CHAPTER 3:

Genetic diversity and population differentiation of the non-indigenous fish, *Gambusia holbrooki*, in Australia

3.1 Introduction

The small ovoviviparous fish, *Gambusia holbrooki,* is a native of southern north America (Rivas 1963; Wooten *et al.* 1988). They are a highly fecund and extremely hardy fish that thrives in a wide range of environmental conditions (Arthington 1989; Arthington and Lloyd 1989; Haynes and Cashner 1995; Hubbs 1999; Kandl 2001; Keane 2002). *Gambusia holbrooki* and its sister species *G. affinis* are otherwise known as mosquitofish and, as the name suggests, sometimes feed on mosquitoes (Lloyd 1990b). For this reason *Gambusia* have been extensively introduced and distributed to many countries around the world (Wilson 1960; Courtenay and Meffe 1989; Gutierrez-Estrada *et al.* 1998; Economidis *et al.* 2000; Garcia-Berthou *et al.* 2005). Unfortunately, the effectiveness of *Gambusia* as a mosquito control agent has been, at best, questionable (Lloyd 1990b; Garcia-Berthou 1999).

Mosquitofish are considered a pest in most locations outside of their native range. These fish are of particular interest because they are responsible for a range of negative impacts on native species and habitats where they have been introduced. In particular, they have been implicated in the decline of many frog (Lawler *et al.* 1999; Komak and Crossland 2000; Hamer *et al.* 2002; Kats and Ferrer 2003) and fish species (Lloyd 1990a; Howe *et al.* 1997; Ivanstoff and Aarn 1999; Morgan *et al.* 2004) around the world.

In Australia, *Gambusia* were first introduced in the early 1900's (Wilson 1960). Since that time, their distribution has been facilitated by the defence forces and municipal councils in the eastern states and South and Western Australia (Clunie *et al.* 2002). *Gambusia* are currently well established throughout Queensland, NSW and the entire Murray Darling Basin (Clunie *et al.* 2002). They can be found in all coastal drainage

systems in Victoria and the entire Gulf drainage division and parts of the Western plateau and Lake Eyre drainages in South Australia (Arthington and Lloyd 1989). In Western Australia, they are currently widely distributed in the south west corner of the state and in the southern Pilbara (Morgan *et al.* 2004).

Mosquitofish were not introduced into the island state of Tasmania until 1992, when approximately fifty fish were deliberately and illegally introduced into a private farm dam at Legana, northern Tasmania (IFS, *unpublished data*). The fish were believed to have been sourced from southeast Queensland (see Chapter 1). Several eradication attempts occurred in the following years and *Gambusia* were, at that time, thought to be restricted to two small private dams at Legana (IFS *unpublished data*). However, in 2000, *Gambusia* were discovered in the Tamar Island Wetlands Reserve, approximately 8 km south-west (upstream) of the original introduction site (Keane 2002; Keane and Neira 2004). Anecdotal information indicates that fish were introduced to TIWR around the same time as the Legana introduction (see Chapter 1) and populations have since been discovered at several sites adjacent to the estuary along an 18 km stretch of the upper Tamar Estuary (see Chapter 2). Several studies have examined diversity and genetic structure of *Gambusia* populations (Brown 1985, 1987; Smith *et al.* 1989; Congdon 1995). In their native range, mosquitofish populations are generally characterized by high diversity and extensive population subdivision over small spatial scales (Brown 1985; Wooten *et al.* 1988; Smith *et al.* 1989; Congdon 1995). However, introduced populations have been generally found to exhibit low allozyme diversity while maintaining high levels of population subdivision (Hughes *et al.* 1991; Congdon 1992, 1995), making it difficult to examine patterns of diversity or infer patterns of dispersal in non-native Australian populations.

The relatively recent and well documented introduction of *Gambusia* into Tasmania provided a unique opportunity to examine *Gambusia* population diversity and dispersal during the early establishment-phase of the introduction. In this chapter, the patterns of diversity and genetic structure of Tamar Estuary *Gambusia* populations are examined using hyper-variable microsatellite DNA loci. This work aims to 1) determine whether the long history of human-mediated dispersal in Australia has reduced the genetic diversity Australian *Gambusia* populations, 2) confirm the alleged source of the Tasmanian fish by comparing Tasmanian *Gambusia* populations with example populations from mainland Australia, and 3) identify the core populations driving dispersal of *Gambusia* in the upper Tamar Estuary, northern Tasmania.

3.2 Materials and methods

3.2.1 Sample collection

Annual surveys for *Gambusia holbrooki* were carried out since 2004 (see Chapter 2) and populations have been discovered at several sites within an 18 km stretch of the upper Tamar Estuary (Fig. 3.1). Tasmanian samples were collected during annual distribution surveys conducted over three consecutive summers during the period 2004-2006 (Table 3.1). Population samples of *Gambusia holbrooki* (n=528) from Tasmanian populations and a single Queensland population were collected using a fine mesh dip-net, stored in 70-95% ethanol directly after capture, and later preserved in 95% ethanol. Population samples from single sites in Western Australia, South Australia and Victoria were collected and donated by researchers in each State (Table 3.1; Fig. 3.1) and

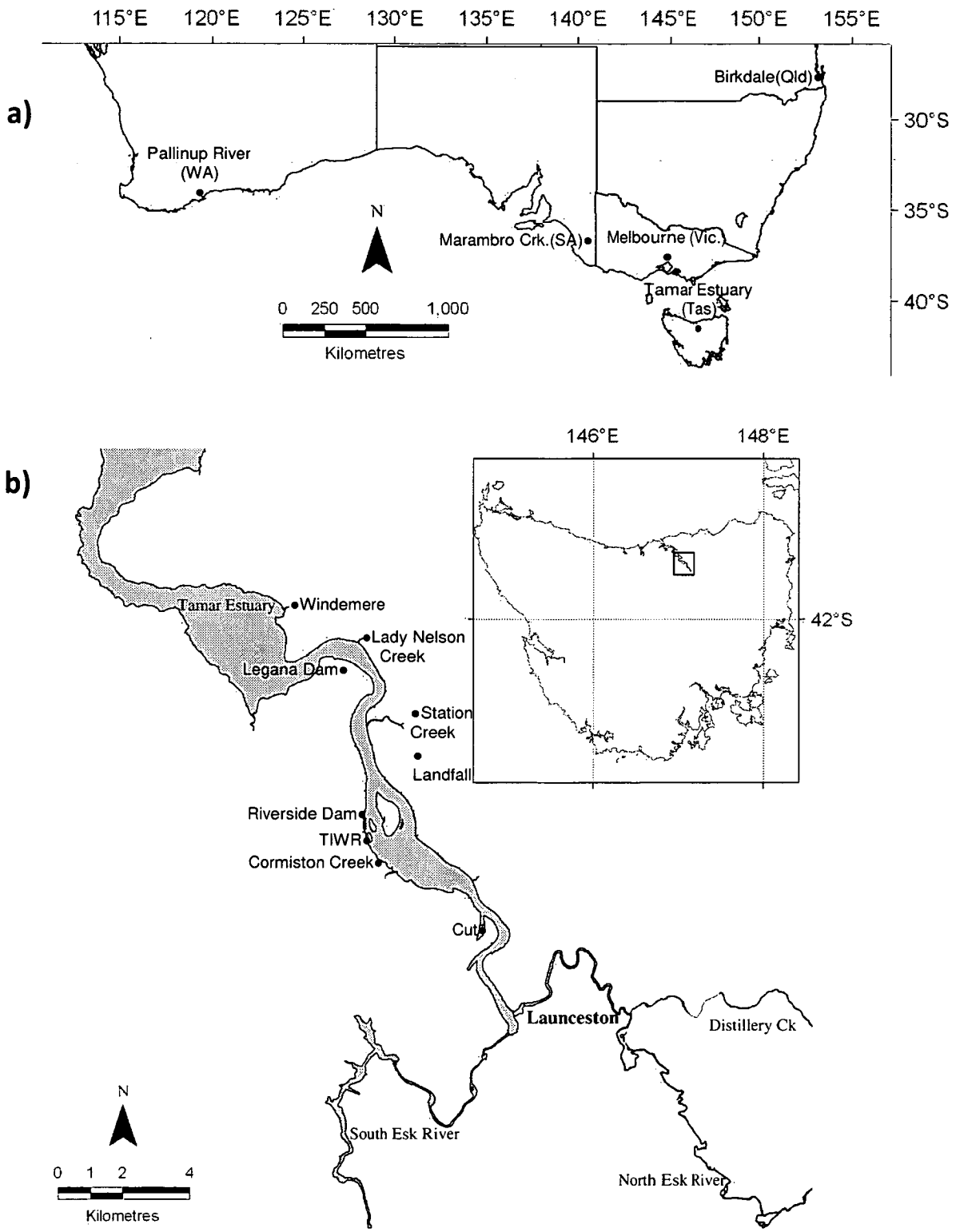

Figure 3.1. Geographic location of *G. holbrooki* collection sites on a) mainland Australia, and b) Tamar Estuary region in northern Tasmania (inset).

Table 3.1. Site descriptions and geographical location of *Gambusia holbrooki* collection sites. Habitat types: A, artificial shallow freshwater impoundment; B, open wetland subject to regular tidal incursions; C, small estuarine creek; D, artificial network of drainage channels; E, modified creek with seasonal freshwater flow and regular tidal incursions; F, small freshwater creek with variable flow regime; G, large river with seasonally variable flow.

Site Name	Code	Description	Location	Grid Ref	Collection date	Sample size (*n*)
Legana Dam	LD2	A	Approx. 15 km north of Launceston, TAS	41° 20' 58.0 " S 147° 03' 7.91" E	Feb 2005	53
Riverside Dam	R	A	Approx. 9 km north of Launceston, TAS	41° 22' 6.83" S 147° 04' 17.3" E	Feb 2005	49
Tamar Island Wetland Reserve (TIWR)	TIWR	B	Approx. 7.5 km north of Launceston, TAS	41° 23' 54.5 " S 147° 04' 34.5 " E	Feb 2005	48
Cormiston Creek	CC	C	Approx. 7 km north of Launceston, TAS	41° 23' 9.42 " S 147° 04' 8.50 " E	Mar 2004	16
Landfall	LF	D	Approx. 9 km north of Launceston, TAS	41° 22' 00.3" S 147° 05' 7.24" E	Feb 2005	48
Station Creek	SC	E	Approx. 10 km north of Launceston, TAS	41° 21' 18.4" S 147° 05' 34.2" E	Feb 2005	21
Lady Nelson Creek	LN	C	Approx. 14 km north of Launceston, TAS	41° 19' 9.12" S 147° 04' 28.7" E	Mar 2004	10
The Cut	CUT	D	Approx. 3 km north of Launceston, TAS	41° 24' 36.6 " S 147° 07' 4.5 " E	Feb 2006	45
Windemere	W	B	Approx. 16 km north of Launceston, TAS	41° 18' 52.6" S 147° 02' 57.9" E	Feb 2006	51
Queensland	QLD	A	Birkdale, QLD	27° 30' 06.4" S 153° 12' 45.7" E	Mar 2006	48
Victoria	VIC	A	North Melbourne, VIC	37° 47' 31.01" S 145° 14' 34.85" E	June 2005	50
South Australia	SA	F	25 kms north of Naracoorte, SA	36° 44' 59.77" S 140° 34' 22.36" E	June 2005	50
Western Australia	WA	G	Pallinup River, WA	34° 24' 27.41" S 118° 43' 46.76" E	Feb 2005	39

preserved as above. Considerable effort was made to collect a sample of at least 50-100 individuals at each site, however this could not be achieved at sites with extremely low population densities (Table 3.1).

3.2.2 DNA isolation, amplification and microsatellite analysis

DNA was isolated from small caudal fin clips taken from ethanol preserved specimens that had been stored from 3 to 18 months. DNA was extracted using the simple "HotSHOT" method involving a 35 minute incubation at 95 °C in alkaline lysis solution followed by addition of a neutralizing solution and storage at -20°C (Truett *et al.* 2000). DNA extracts were stored at -20°C until just prior to use in PCR reactions. Ten microsatellite loci were used that had been developed specifically for use with *Gambusia* spp., Gafμ1 to Gafμ7 for *Gambusia affinis* and M*f*1, M*f*6 and M*f*13 for *G. holbrooki* (Spencer *et al.* 1999; Zane *et al.* 1999). Pilot studies with a limited number of fish revealed that some loci contained null alleles, or were difficult to interpret and these loci were excluded from further use. Six loci (Gafμ2, Gafμ4 and Gafμ7 and M*f*1, M*f*6 and M*f*13) were selected for examining diversity in *G. holbrooki* in Australia.

Amplification of Gafμ2, Gafμ4 and Gafμ7 loci were performed in 15 μl reaction volumes containing 5 μl of genomic DNA extract, 0.6 pmol end-labeled forward primer and unlabelled reverse primer, 100 μM each dNTP, 1.5 mM $MgCl_2$, 0.6 U of BIOTAQ™ DNA polymerase, and 1.5μl of 10x PCR buffer (consisting of 160 mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20). Amplification and cycling of loci M*f*1, M*f*6 and M*f*13 followed conditions given by (Zane *et al.* 1999). Locus M*f*6 and Locus M*f*13 were multiplexed. The cycling conditions for all Gafμ loci followed the

protocol described by (Spencer *et al.* 1999). All loci were amplified using an Eppendorf gradient mastercycler. Fluorescently labeled PCR products were size-separated and analysed using a Beckman-Coulter CEQ 8000™ genetic analysis system and software package. Genotypes were assigned to each individual fish based on the size of the PCR products (base pairs) observed in the electropherogram.

3.2.3 Statistical Analyses

3.2.3.1 Genetic diversity within populations

Genetic diversity measures, allelic diversity (*A*), and observed (H_o)and expected (H_e) heterozygosity were calculated using the software program GenAlEx ver. 6.0 (Peakall and Smouse 2005). Comparisons of allelic diversity can be biased when there are differences in sampling intensity (Leberg 2002). To account for variation in sample sizes, *A* was standardized via rarefaction using the program FSAT (Goudet 2001). Differences in adjusted *A* among populations was tested using a single factor analysis of variance (ANOVA) with the statistical program SPSS (ver. 14.0 for windows, SPSS Inc.). The non-parametric Kruskal-Wallis test was used to test for significance of variation in expected levels of heterozygosity (H_e) among populations. Evidence of linkage disequilibrium and deviations from Hardy-Weinberg equilibrium were assessed using Markov chain approximations (dememorization = 10000, batches = 100, 5000 iterations per batch) in the program GENEPOP (Raymond and Rousset 1995). Evidence of genotyping errors in the data set were assessed using the program MICRO-CHECKER (Oosterhout *et al.* 2004). Genotypic linkage disequilibrium was tested for all locus/population combinations

by unbiased estimates of Fishers' exact tests using the Markov chain method in GENEPOP. To compensate for multiple statistical tests, levels of significance were adjusted using the Bonferroni method (Rice 1989).

3.2.3.2 Population differentiation

Population differentiation was assessed using analysis of molecular variance (AMOVA) to calculate the variance within and between populations/regions (Excoffier *et al.* 1992). The AMOVA framework in GenAlEx6 was utilized to perform hierarchical analysis of genetic differentiation with tests of significance via random permutation (Peakall and Smouse 2005). Data were divided into 5 regions (WA, SA, Victoria, Queensland, and Tasmania) for the AMOVA analysis. The proportion of genetic variation was determined among populations (F_{ST}), among populations within regions (F_{SR}) and among regions (F_{RT}). Pairwise F_{ST} values were calculated using GenAlEx6 and tested for significance using 9999 random permutations of the data set. Finally, gene frequency data were used to generate 1000 bootstapped genetic distance matrices using Nei's unbiased genetic distance (Nei 1972) in PHYLIP (Felsenstein 2006). These distance matrices were used to generate 1000 neighbour-joining trees using NEIGHBOR program in PHYLIP. Trees were then summarized using the CONSENSE program in PHYLIP.

The genetic population structure of the 13 *Gambusia holbrooki* populations was further investigated using the Bayesian clustering algorithm in the program STRUCTURE version 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003). STRUCTURE places individuals into (K) genetically distinct clusters, without a priori population information. To determine the optimal value of K, the data was run through the program with varying burn-in lengths

and several runs at each value of K (varying from 1 to13). The program was run under the admixture model, the length of the initial burn-in was set at 200000 iterations followed by a run of 200000 Markov chain Monte Carlo (MCMC) repetitions. The appropriate value of K was determined using the method outlined in the program documentation (Pritchard *et al.* 2007).

Assignment testing using the Bayesian (Rannala and Mountain 1997) and frequencies-based methods (Paetkau *et al.* 2004) of analysis were employed using the software program GeneClass2 (Piry *et al.* 2004). The purpose of this analysis was to identify for each individual of a population the probability that it belongs only to that population, is a migrant from each of the other populations, and the probability of it being a migrant to other populations. For each run, 10000 genotypes were simulated for each population and a threshold probability value of 0.01 was applied to determine the origin.

3.2.3.3 Patterns of genetic differentiation

Allele frequency variation at all loci across all populations was summarized in two dimensions using Multidimensional Scaling Analysis (MDS). The analysis was carried out using the software program SPSS (ver. 14.0 for windows, SPSS Inc.) using the allele frequency matrix generated in GenAlEx6.

To test for isolation by distance among Tasmanian populations, the relationship between the pairwise matrix of genetic distance [FST/(1-FST)] and a log-transformed matrix of geographic distances was compared using a Mantel test in GenAlEx6 (10,000 permutations). Geographic distance between Tasmanian populations was measured as estimated estuary distance in kilometres determined using the measure function in

ArcView (GIS ver 3.3 Environmental Science Research Institute, 2002).

3.3 Results

3.3.1 Genetic diversity within populations

Of the six loci used to assess genetic diversity of *Gambusia*, two (M*f*1 & M*f*6) were monomorphic across all populations. Gafμ4 was the most diverse locus, with 13 alleles across all the populations assayed (Table 3.2). Of the four polymorphic loci used in this study, Queensland had 8 alleles, Western Australia had 17, South Australia had 15, and Victoria had 7. The total number of alleles varied from 7 to 11 among Tasmanian populations, with R and TIWR highest (11 alleles), followed by W (10 alleles)(Table 3.2). The allele (155) at locus Gafμ2 was rare among Tasmanian *Gambusia* populations and was only found in fish collected from two sites (LD2 and TIWR), while it occurred in varying frequencies in all mainland populations (Table 3.2). At locus Gafμ7, (189) was the most common allele across all populations with the exception of VIC and SA. Of the mainland populations, QLD was most similar to Tasmanian populations with (189) being the most common allele at locus Gafμ7 and allele (241) occurring at much lower frequency (Table 3.2). Patterns in allele frequencies among populations were less obvious at the most diverse locus (Gafμ4). However, the allele (250) was found in all Tasmanian and none of the mainland populations (Table 3.2).

Table 3.2. Summary table of allele frequency and locus by locus genetic variability estimates of *Gambusia* populations. Number of Alleles (N_a) and adjusted N_a (adj) via rarefaction, observed (H_o) and expected (H_e) heterozygosity and the number of private alleles (#PA) averaged across all loci per population. TAS comb represents all of the Tasmanian populations pooled and shaded columns are Tasmanian populations.

Locus			LD2	R	TIWR	CC	LF	SC	LN	CUT	W	QLD	VIC	SA	WA	TAS comb	All Pops
Mf13	alleles	156	0.663	0.776	0.585	0.594	0.146	0.190	0.722	0.453	0.500	0.717	0.958	0.633	0.244		
		162	0.337	0.224	0.415	0.406	0.854	0.810	0.278	0.547	0.500	0.283	0.042	0.367	0.756		
	Na		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Na (adj)		1.99	1.96	1.99	2	1.86	1.95	1.99	2	2	1.98	1.41	1.99	1.97		1.99
	# reps		52	49	47	16	48	21	9	43	49	46	48	49	39	334	516
	H_e		0.447	0.348	0.486	0.42	0.249	0.308	0.401	0.496	0.5	0.405	0.08	0.465	0.369	0.5	0.387
	H_o		0.481	0.245	0.447	0.313	0.292	0.381	0.556	0.581	0.551	0.435	0.083	0.49	0.333	0.425	0.399
	#PA		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gafμ2	alleles	151	0.530	0.894	0.862	0.643	0.837	0.714	0.850	0.289	0.765						
		153	0.410	0.106	0.106	0.357	0.163	0.286	0.150	0.711	0.235	0.990		0.120	0.789		
		155	0.060		0.032							0.010	1.000	0.880	0.211		
	Na		3	2	3	2	2	2	2	2	2	2	1	2	2	3	3
	Na (adj)		2.54	1.76	2.1	1.99	1.89	1.99	1.95	1.98	1.96	1.12	1	1.8	1.95		2.93
	# reps		50	47	47	14	46	21	10	45	51	48	50	50	38	331	517
	H_e		0.547	0.19	0.245	0.459	0.273	0.408	0.255	0.411	0.36	0.021	0	0.211	0.332	0.427	0.286
	H_o		0.48	0.213	0.213	0.429	0.283	0.476	0.3	0.4	0.353	0.021	0	0.24	0.421	0.338	0.294
	#PA		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Gafμ7	alleles	175												0.140			
		189	0.740	0.857	0.896	1.000	1.000	1.000	0.938	1.000	0.833	0.989	0.184	0.150	0.878		
		193												0.380	0.068		
		199												0.010			
		205											0.816	0.300	0.054		
		209												0.020			
		241	0.260	0.143	0.104				0.063		0.167	0.011					
	Na		2	2	2	1	1	1	2	1	2	2	2	6	3	2	7
	Na (adj)		1.97	1.86	1.75	1	1	1	1.75	1	1.9	1.13	1.92	4.06	2.11		2.90
	# reps		50	49	48	16	47	21	8	45	51	46	49	50	37	335	517
	H_e		0.385	0.245	0.187	0	0	0	0.117	0	0.278	0.022	0.3	0.723	0.221	0.182	0.191
	H_o		0.28	0.286	0.208	0	0	0	0.125	0	0.255	0.022	0.327	0.66	0.243	0.155	0.185
	#PA		0	0	0	0	0	0	0	0	0	0	0	3	0	0	3

Table 3.2. (cont.)

Locus			LD2	R	TIWR	CC	LF	SC	LN	CUT	W	QLD	VIC	SA	WA	TAS comb	All Pops
Gafμ4	alleles	236											0.541	0.060			
		246													0.051		
		250	0.382	0.449	0.417	0.154	0.761	0.738	0.333	0.302	0.049						
		254													0.026		
		256		0.347	0.125	0.385	0.239	0.262			0.647	0.135	0.459	0.250	0.026		
		258		0.173	0.292	0.231			0.667	0.698	0.088				0.487		
		262	0.618	0.020	0.167	0.231					0.216	0.865		0.540	0.051		
		266		0.010										0.010	0.256		
		268													0.038		
		276												0.140			
		278													0.026		
		280													0.013		
		282													0.026		
	Na		2	5	4	4	2	2	2	2	4	2	2	5	10	5	13
	Na (adj)		1.99	3.26	3.71	3.907	1.97	1.98	2	1.99	3.11	1.84	2	3.49	4.66		4.83
	# reps		51	49	48	13	46	21	6	43	51	48	49	50	39	328	514
	H_e		0.472	0.647	0.698	0.722	0.364	0.387	0.444	0.422	0.525	0.234	0.497	0.623	0.687	0.718	0.517
	H_o		0.569	0.347	0.521	0.308	0.087	0.048	0.333	0.279	0.431	0.271	0.469	0.7	0.641	0.354	0.385
	#PA		0	0	0	0	0	0	0	0	0	0	0	1	6	1	7
Average (Total)																	
	Na		2.25 (9)	2.75 (11)	2.75 (11)	2.25 (9)	1.75 (7)	1.75 (7)	2 (8)	1.75 (7)	2.5 (10)	2 (8)	1.75 (7)	3.75 (15)	4.25 (17)	3 (12)	2.42 (25)
	Na (adj)		2.12	2.21	2.39	2.22	1.68	1.73	1.92	1.74	2.24	1.52	1.58	2.84	2.67		3.16
	# reps		50.75	48.5	47.5	14.75	46.75	21	8.25	44	50.5	47	49	49.75	38.25	332	516
	H_e		0.463	0.358	0.404	0.416	0.221	0.276	0.304	0.332	0.416	0.187	0.219	0.505	0.402	0.457	0.345
	H_o		0.452	0.273	0.347	0.262	0.165	0.226	0.328	0.315	0.398	0.17	0.220	0.522	0.41	0.318	0.316
	#PA		0	0	0	0	0	0	0	0	0	0	0	(4)	(6)	(2)	(10)

Uncorrected allelic diversity (A) was highest in Western Australia (4.25) and the lowest (1.75) was shared by Victoria, and three Tasmanian populations (Table 3.3). Overall, the mainland populations of South Australia and Western Australia had a greater number of alleles per locus than Tasmanian and Victorian populations (Table 3.3), however, when allelic diversity was corrected for sample sizes, there were no significant differences among populations (ANOVA, $P = 0.303$). Similarly, no significant difference was found in H_e among populations (Kruskal-Wallis $P = 0.457$).

Only two of the 13 populations examined in this study had private alleles (alleles unique to a single population) (Table 3.3). The Western Australian population of *Gambusia* contained six private alleles, South Australia had 4 and none were recorded in any of the individual Tasmanian populations (Table 3.3). However, when combined, the Tasmanian populations of *Gambusia* contained two unique alleles not present in any of the mainland populations (Table 3.2).

After Bonferroni correction significant departures from Hardy-Weinberg equilibrium (HWE) and an excess of homozygotes was detected in five Tasmanian populations; R, CC, LF, SC and W (Table 3.3). There were no departures from HWE in any of the mainland populations of *Gambusia* (Table 3.3). MICROCHECKER analysis showed significant departures from HWE in five Tasmanian populations; R, CC, LF, SC and TIWR. All of the departures occurred at the locus Gafμ4 and there was evidence of a null allele in each case. Tests for linkage disequilibrium among pairs of loci were all non-significant following Bonferroni correction.

Table 3.3. Summary of microsatellite diversity of 13 *Gambusia holbrooki* populations. Microsatellite diversity, heterozygosity and Hardy-Weinberg (HWE) conformity calculated for each population of *Gambusia holbrooki*. Allelic diversity (*A*) is given as direct count of the average number of alleles per locus and as adjusted (*A*), where sample size variation has been corrected via rarefaction. Heterozygosity estimates for each population are given as observed (H_o) and expected (H_e). Populations in Hardy-Weinberg equilibrium are represented as Eq, while **Ex** indicates non-conformity to HWE and an excess of homozygotes in the population. Bonferroni correction ($P < 0.0125$) was applied to significance tests across all loci. Tasmanian populations are shaded.

Population	Mean size	Allelic diversity (*A*)	Adjusted (*A*)	H_o	H_e	#Private alleles	HWE
LD2	50.75	2.25	2.12	0.452	0.463	0	Eq
R	48.5	2.75	2.21	0.273	0.358	0	**Ex**
TIWR	47.5	2.75	2.39	0.347	0.404	0	Eq
CC	14.75	2.25	2.22	0.262	0.416	0	**Ex**
LF	46.75	1.75	1.68	0.165	0.221	0	**Ex**
SC	21	1.75	1.73	0.226	0.276	0	**Ex**
LN	8.25	2	1.92	0.328	0.304	0	Eq
CUT	44	1.75	1.74	0.315	0.322	0	Eq
W	50.5	2.5	2.24	0.398	0.416	0	**Ex**
QLD	47	2	1.52	0.17	0.187	0	Eq
VIC	49	1.75	1.58	0.220	0.219	0	Eq
SA	49.75	3.75	2.84	0.522	0.505	4	Eq
WA	38.25	4.25	2.67	0.41	0.402	6	Eq

3.3.2 Population differentiation

Hierarchical AMOVA analysis revealed significant genetic variation among regions (Australian states) (F_{RT} = 0.343, $P < 0.01$, 34%), among populations within regions (F_{SR} = 0.227, $P < 0.01$, 15%) and among populations (F_{ST} = 0.492, $P < 0.01$, 51%). Levels of population differentiation (F_{ST}) were generally high and ranged from 0.001 to 0.748 (Table 3.4). Only one pairwise comparison, between the two Tasmanian populations SC and LF, was non-significant (Table 3.4). These two populations are situated in close proximity to one another (Fig. 3.1). All comparisons involving Lady Nelson Creek (LN) and Cormiston Creek (CC) should be viewed with caution due to extremely low sample sizes.

In the dendrogram constructed using Nei's genetic distance, the Victorian and South Australian populations formed a well supported group (Fig. 3.2). The node separating Queensland, WA and the two Tasmanian populations (LD2 and CUT) from the rest of the Tasmanian populations was weakly supported (bootstrap 25). Among the Tasmanian populations, LF and SC fall out distinctly from the others (bootstrap 94). A core group of Tasmanian populations is evident (R, TIWR, LN, CC, and W), but with weak bootstrap support (Fig. 3.2).

The analysis of the *13 Gambusia holbrooki* populations using the program STRUCTURE indicated that the overall genetic profile could be best described in six (K=6) hypothetical clusters (Table 3.5). The average values of ancestry probabilities of each population in the six clusters are presented in Table 3.5. The first 3 clusters (K=1-3) generally consist of a mix of Tasmanian populations, indicating a high degree of coancestry (Table 3.5). The population sampled in Queensland had a very high

probability of membership to only one cluster (0.913 in cluster 4) and coancestry only with fish sampled at LD2. Similarly, WA has a high probability of belonging to cluster 5 (0.891) and coancestry with CUT (0.542) (Table 3.5). The final cluster (6) had a very high probability of containing members only from SA and VIC (Table 3.5).

The Bayesian method of (Rannala and Mountain 1997) assignment performed better (62.7%) than the frequencies based method (52.7%)(Paetkau *et al.* 2004) in assigning individuals to correct sampling locations. The proportion of immigrants (*m*) into each population, estimated by GENECLASS2 (Piry *et al.* 2004) are presented in Table 3.6. In the matrix, the values in the diagonal represent the proportions of individuals derived from the same population as that from which they were sampled. The values are fairly consistent across all populations with the lowest at CC (0.248) and the highest at LF (0.487) (Table 3.6). Multidirectional migration rates among Tasmanian populations were high, as evidenced by the many bold estimates in the matrix (Table 3.6). As stated previously, it is important to note that the results relating to the sites LN and CC are based on very small sample sizes and should be viewed with caution. Migration both into, and primarily, out of TIWR was very high (Table 3.6). Migration from TIWR occurred towards all other Tasmanian populations (see highlighted area on Table 3.6). Migration rates between mainland and other populations was limited. However, there was evidence of migration between CUT and WA, and LD2 and QLD respectively (Table 3.6).

3.3.3 Patterns of genetic differentiation

Allele frequency differences represented in the MDS generally reflected the relationships detected by Nei's genetic distance, STRUCTURE analysis and assignment

testing (Fig. 3.3). Tasmanian populations clustered together, and QLD and WA are more similar to Tasmanian populations than SA and Victoria (Fig. 3.3). The core cluster of Tasmanian populations identified in the dendrogram was evident, with LD2 and CUT appearing to be outliers. No significant relationship was observed between geographic and genetic distance among Tasmanian populations (Mantel, $R^2 = 0.049$, $P = 0.149$).

Table 3.4. Pairwise estimates of genetic differentiation (F_{ST}) among 13 Australian *Gambusia* populations. Non significant values are shaded and probability values derived from 9999 permutations are given above the diagonal.

	LD2	R	TIWR	CC	LF	SC	LN	CUT	W	QLD	VIC	SA	WA
LD2		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
R	0.185		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
TIWR	0.132	0.041		0.003	0.001	0.001	0.046	0.001	0.001	0.001	0.001	0.001	0.001
CC	0.116	0.090	0.057		0.001	0.001	0.002	0.001	0.022	0.001	0.001	0.001	0.001
LF	0.330	0.289	0.200	0.295		0.363	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SC	0.258	0.242	0.162	0.205	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001
LN	0.212	0.094	0.037	0.112	0.399	0.332		0.001	0.001	0.001	0.001	0.001	0.001
CUT	0.272	0.328	0.239	0.187	0.406	0.320	0.203		0.001	0.001	0.001	0.001	0.001
W	0.188	0.126	0.124	0.036	0.281	0.224	0.217	0.312		0.001	0.001	0.001	0.001
QLD	0.266	0.547	0.497	0.415	0.679	0.633	0.629	0.446	0.450		0.001	0.001	0.001
VIC	0.561	0.603	0.604	0.618	0.748	0.721	0.681	0.671	0.579	0.741		0.001	0.001
SA	0.328	0.453	0.422	0.374	0.561	0.495	0.453	0.481	0.394	0.485	0.296		0.001
WA	0.316	0.434	0.351	0.258	0.460	0.368	0.358	0.124	0.341	0.404	0.629	0.400	

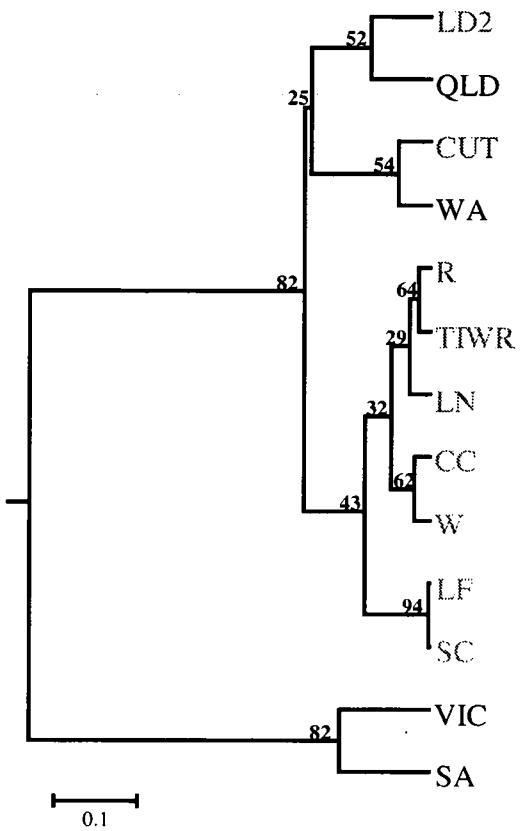

Figure 3.2. Dendrogram of UPGMA clustered *Gambusia* populations based on Nei's unbiased genetic distance. Tasmanian populations are represented in grey font. Number at nodes indicates the percentage support value from 1000 bootstrap replicates.

Table 3.5. Average coefficients of ancestry obtained from a STRUCTURE run with *K*=6, for the 13 *Gambusia holbrooki* populations analysed. The highest values of coancestry of each population in a cluster is in bold. #Indiv. is the number of individuals in each cluster.

	Cluster (*K*)						
	1	2	3	4	5	6	# Indiv.
LD2	**0.497**	0.05	0.151	**0.228**	0.04	0.034	53
R	**0.299**	**0.394**	**0.228**	0.02	0.048	0.01	49
TIWR	**0.489**	0.163	**0.228**	0.031	0.076	0.014	48
CC	0.16	**0.318**	0.189	0.178	0.144	0.011	16
LF	0.046	0.24	**0.658**	0.02	0.029	0.008	48
SC	0.056	0.263	**0.627**	0.022	0.026	0.007	21
LN	**0.558**	0.147	0.183	0.025	0.076	0.011	10
CUT	**0.211**	0.03	0.168	0.042	**0.542**	0.007	45
W	**0.201**	**0.635**	0.037	0.061	0.055	0.01	51
QLD	0.02	0.02	0.01	**0.913**	0.025	0.011	48
VIC	0.009	0.02	0.008	0.011	0.009	**0.943**	50
SA	0.016	0.015	0.012	0.073	0.03	**0.854**	50
WA	0.017	0.017	0.011	0.032	**0.891**	0.033	39

Table 3.6. Means of migration rate (*m*) into (rows) and from (columns) each population as calculated by GENECLASS2. In rows, the populations from which the individuals probably belong to the reference population as in columns. Shaded cells are the proportions of individuals derived from the source population. Values of *m* above 0.100 are in bold. Broken border illustrates migration from TIWR.

	LD2	R	TIWR	CC	LF	SC	LN	CUT	W	QLD	VIC	SA	WA
LD2	**0.429**	0.079	**0.229**	**0.107**	0.012	0.019	0.028	0.016	0.085	0.031	0.001	0.015	0.008
R	0.069	**0.442**	**0.396**	**0.211**	0.059	0.074	**0.106**	0.036	**0.228**	0.001	0.000	0.001	0.005
TIWR	**0.128**	**0.278**	**0.426**	**0.189**	0.048	0.059	**0.104**	0.035	**0.161**	0.000	0.000	0.000	0.016
CC	**0.129**	**0.254**	**0.271**	**0.248**	**0.136**	**0.173**	**0.117**	**0.136**	**0.377**	0.086	0.000	0.008	0.081
LF	0.095	**0.250**	**0.453**	**0.111**	**0.487**	**0.583**	0.063	0.027	**0.167**	0.001	0.000	0.001	0.017
SC	**0.138**	**0.223**	**0.388**	0.097	**0.337**	**0.453**	0.062	0.035	**0.174**	0.000	0.000	0.000	0.000
LN	**0.247**	**0.355**	**0.545**	**0.333**	0.058	**0.100**	**0.361**	**0.138**	**0.259**	0.000	0.000	0.000	0.000
CUT	0.068	0.079	**0.193**	**0.106**	0.029	0.055	**0.111**	**0.486**	0.035	0.007	0.000	0.000	**0.271**
W	0.058	**0.194**	**0.199**	**0.294**	0.025	0.032	0.002	0.000	**0.472**	0.000	0.000	0.000	0.027
QLD	**0.260**	0.005	0.012	0.062	0.001	0.001	0.000	0.000	0.068	**0.469**	0.000	0.017	0.079
VIC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.486**	**0.175**	0.000
SA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	**0.459**	0.002
WA	0.000	0.000	0.003	0.001	0.000	0.000	0.002	0.036	0.000	0.000	0.000	0.000	**0.448**

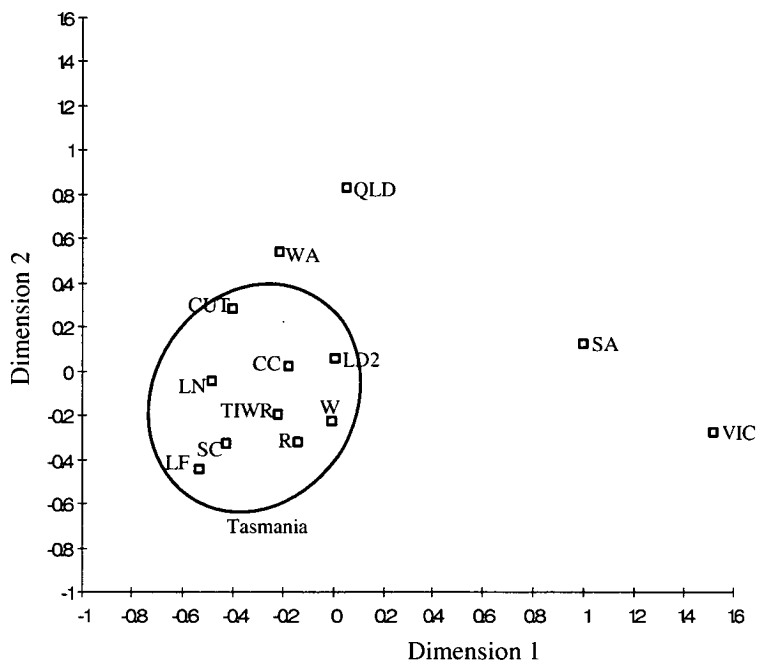

Figure 3.3. Multidimensional scaling analysis from allele frequency data of each population (stress = 0.085). A geographical overlay of sites indicates the grouping of Tasmanian versus mainland populations.

3.4 Discussion

3.4.1 Genetic diversity of Australian Gambusia

In this study there was no significant difference in allelic diversity among any of the sampled populations. The statistical power to detect any differences would have been enhanced if more than four loci could have been utilized. Regardless of this shortcoming, the allelic diversity of the Australian *Gambusia* populations examined in this study is low compared to native range populations from southern United States. (Zane *et al.* 1999) used microsatellite markers M*f*1, M*f*6 and M*f*13 to examine paternity in *G. holbrooki* from two native range populations in South Carolina (USA). While allelic diversity (A) was not specifically presented it could be calculated from the data to provide a direct comparison. Sixteen alleles were encountered across the three loci compared to only four alleles in Australian *Gambusia* across the same three loci, with both Mf1 and Mf6 being monomorphic. Both US populations were more diverse (A= 4.6-5.0) compared to the Australian population as a whole (A=1.3). Similar loss of diversity was also reported for introduced European *Gambusia* compared to home range (Florida) populations using RAPD markers (Grapputo *et al.* 2006).

Loss of diversity can lead to morphological, physiological and behavioural changes in small populations (Lande and Barrowclough 1987; Barrett and Kohn 1991; Tsutsui *et al.* 2000). Invasions of non-native species are characterized by introductions of generally a small number of individuals that are subject to founder effects immediately following introduction (Sakai *et al.* 2001; Allendorf and Lundquist 2003). For this reason, we would expect populations of new invaders to be less genetically diverse than their source population (Barrett and Kohn 1991). Indeed, there are many examples of reduced

diversity in introduced populations (Tsutsui *et al.* 2000; Lasota *et al.* 2004; Grapputo *et al.* 2005; Lindholm *et al.* 2005; Grapputo *et al.* 2006). It is difficult to explain why so many organisms are able to successfully invade new habitats when small population size often negatively affects population viability. In the case of the invasive Argentine ant, *Linepithema humile*, reduced genetic diversity has been shown to enhance the species ability to invade (Tsutsui *et al.* 2000). In its native range, *L. humile* coexists with other ants in species rich communities and does not displace or detrimentally affect other species in the community (Tsutsui *et al.* 2000). However, loss of diversity in introduced populations is associated with a behavioural change in territoriality, resulting in the formation of super-colonies. The invasive ant is able to gain numerical superiority and out-compete native ants due to reduced costs in maintaining territoriality. In this study, the data from *Gambusia* indicate that invasion success, even in early expansion phase examined here, is not reliant on, or apparently affected by relatively severe reductions in genetic variability. *Gambusia* populations are incredibly resistant to the negative effects of demographic bottlenecks (Chapter 4). Populations subjected to severe bottlenecks that reduce genetic diversity can recover in a relatively short periods of time (i.e. one summer breeding season) (Chapter 4). *Gambusia* populations display highly variable life history characteristics (e.g. fecundity, size at maturity, and the length of the reproductive season) across the native and introduced ranges of the species (Haynes and Cashner 1995). This life history plasticity, coupled with high fecundity (Milton and Arthington 1983), multiple paternity (Zane *et al.* 1999), rapid population growth (Lloyd *et al.* 1986), a generalist diet (Pen *et al.* 1993; Garcia-Berthou 1999), broad physiological tolerances (Pyke 2005), and the ability to thrive in habitats disturbed by humans (Arthington 1990), maximizes maintenance of genetic diversity in *Gambusia* populations and contributes to its success as

an invader. However, despite the vast body of work relating to the fish (*see review by* (Pyke 2005)), further research is still required to isolate the exact mechanism(s) responsible for the remarkable invasion success of *Gambusia.*

3.4.2 Patterns of population differentiation among Australian Gambusia

The data generated from this study indicate that Tasmanian populations are genetically more similar to each other than to mainland populations, and that the pattern of population similarity is consistent with a single introduction event to Tasmania. All of the analyses (i.e. dendrogram, MDS, ancestry and assignment testing) showed evidence supporting this hypothesis. The similarity between Tasmanian *Gambusia* populations and the single QLD population examined here also supports the putative suburban Brisbane source of the *Gambusia* introduced to Tasmania (Figs 3.2, 3.3; Table 3.5). While allele frequency analyses indicate that the WA population is also very similar to Tasmanian populations, the absence of several unique WA alleles among more than 500 Tasmanian *Gambusia* suggests that the WA population is less likely to be the source population. Given that so few mainland populations were sampled in this study and the documented active human-assisted dispersal of Australian *Gambusia,* it is also possible that Tasmanian populations derive from one of many other mainland populations in several states. It should also be noted that the design of this study was not optimal for determination of the mainland source of the Tasmanian populations. Several samples or sites within regions at each mainland location would have been preferable, in order to assess the variation among regions and between sites within regions. This work would have to be conducted in order to definitively confirm the source of Tasmanian *Gambusia.* Another

useful avenue for further research would be to assess mitochondrial DNA variation among Australian *Gambusia.* Although outside of the scope of the current study, in combination with microsatellite DNA analysis, it can be can be very useful in determining the origin of populations (Rollins *et al.* 2006).

Population genetic analysis can potentially answer many key questions in pest management (*see review by* (Rollins *et al.* 2006)). For example, genetic tools have been used to identify the origin of invasive individuals (this study;(Bonizzoni *et al.* 2004), to examine patterns of spread and dispersal (Bryan *et al.* 2005; Schussman *et al.* 2006), rate of movement of individuals among sub-populations (Hoffman *et al.* 2006), number and size of separate introduction events (Goodisman *et al.* 2001; Grapputo *et al.* 2005), and number of genetically distinct populations and the size of invading populations (this study;(Baker and Clapham 2004; Burns *et al.* 2004; Hoffman *et al.* 2006; Rollins *et al.* 2006). This key information contributes to the understanding of the dynamics of invasions and improves management strategies for individual species. The present study contributes important information regarding the recent invasion of *Gambusia* in Tasmania and demonstrates the utility of MSAT loci in identifying introduction sources and dispersal patterns in species with reduced diversity.

3.4.3 Differentiation and dispersal among Tasmanian populations

Despite the evidence of migration between Tasmanian *Gambusia* populations, the fish are not dispersing with ease or in large numbers throughout the Tamar Estuary region. This hypothesis is supported by the high levels of population differentiation and evidence of population bottlenecks (e.g. excess of homozygotes in this study) that were

detected among Tasmanian populations. In addition, spread has been slow and several populations failed to establish in the long term (Chapter 2), indicating that the species has not established without difficulty. Significantly different allele frequencies across most sites indicates that migration geneflow even between relatively close sites is very restricted. Factors such as physical barriers, unsuitable habitat and distance are significant obstacles for *Gambusia*'s dispersal in the region and explain the relatively slow rate of spread. The similarity between populations at the sites LF and SC indicates a single dispersal event followed by localized spread along interconnected habitat.

Freshwater fish generally exhibit high levels of genetic structuring due to the presence of physical barriers and the ephemeral nature of many habitats (Vrijenhoek 1979). *Gambusia* are no exception and allozyme studies of US populations have also detected significant differentiation among populations at a range of spatial scales (100's m – 100's km) (Kennedy *et al.* 1986; Smith *et al.* 1989; Wooten and Lydeard 1990). Despite the low diversity of Australian *G. holbrooki* populations, significant differentiation could be detected throughout its range in both Tasmania and mainland Australia. (Congdon 1995) found significant genetic structuring in Queensland *G. holbrooki* populations using just one allozyme locus. He found that population differentiation was driven by barriers preventing upstream movement of fish.

In this study, several Tasmanian *Gambusia* populations were not in HWE and were found to have an excess of homozygotes. This could be evidence of inbreeding and bottlenecks, as discussed above. Alternatively, it could be a result of a genotyping error caused by a null allele. The MICROCHECKER analysis indicated the possibility of a null allele in several Tasmanian populations. Null alleles occur when mutations at primer sites cause certain alleles to not amplify. The result of this is false homozygotes (Shaw *et al.* 1999).

This type of genotyping error can lead to deviations from HWE and in particular heterozygote deficiency (Shaw *et al.* 1999), which can potentially bias population genetic analyses. Null alleles are a problem that is more common when using microsatellites from a related species rather than developed for the species in particular (Pemberton *et al.* 1995). All of the deviations from HWE in this study occurred at the locus Gafμ4 which was developed for *Gambusia affinis* rather than *Gambusia holbrooki* (Spencer *et al.* 1999). For this reason the locus should possibly have been deleted from the study. However, as the deviations were not serious and due to the already small number of loci that were successfully analysed it was included in the data set. Any future research on *Gambusia holbrooki* should take this into consideration and attempt to use only those MSAT's specifically designed for the species.

The lack of a significant pattern of isolation-by-distance among Tasmanian populations indicates that there is no clinal pattern to the genetic differentiation between Tamar populations and that physical/biological barriers limiting dispersal are a more important factor structuring genetic variation among populations. Tidal currents, variable freshwater flows and physical structures such as levees and weirs can all act as barriers to *Gambusia* dispersal among suitable habitat along the Tamar Estuary. While tides and freshwater flows could be considered a conduit for dispersal, it is more likely that they act as a barrier due to *Gambusia*'s modest swimming capability and behavioural responses to water flow (Meffe 1984; Congdon 1994; Ward *et al.* 2003). In response to high water velocities mosquitofish will often venture midstream and be displaced (Meffe 1984; Congdon 1994; Ward *et al.* 2003). As a consequence, the tidal dynamics and physical barriers in the estuary are likely to be the dominant forces driving dispersal and genetic structuring of *Gambusia* because they determine which habitats *Gambusia* can access. The

common link between the most differentiated populations (CUT, LD2, SC and LF) in the Tamar is the presence of a physical barrier to fish dispersal. Fish are trapped in these water bodies until high water flows or flood conditions re-connect them to the estuary. LD2, the original introduction site, has been subject to several eradication attempts over the years that create population bottlenecks and lead to drift and shifts in allele frequencies (Chapter 4). The remaining sites would have been colonized by a small number of individuals during flood events. It is likely that very little exchange of individuals with other populations has occurred since colonization due to the presence of physical barriers which would have led to genetic drift and differentiation among Tamar populations.

The genetic evidence of this study suggests that TIWR is primarily driving the spread of *Gambusia* throughout the Tamar Estuary. It has the highest diversity of all Tasmanian sites and the highest levels of migration (to and from). There are high levels of coancestry and migration among other Tasmanian populations such as R, LN, CC, and W. While there is little or no favorable *Gambusia* habitat connecting these populations (and TIWR), they are connected via the tidal Tamar Estuary and there are no permanent physical barriers. Two of these populations (LN and CC) do not appear to have established permanent breeding populations during the course of work in the area (1999-2006) and fish were only ever found in very low abundance at both sites (see Table 2.1; Chapter 2). The failure of *Gambusia* to establish at these sites is almost certainly due to the unsuitability of the habitat as both locations are tidal creeks that can experience very high seasonal water flows. This type of habitat is unsuitable for *Gambusia* as it prefers little or no water flow (Casterlin and Reynolds 1977; Gill *et al.* 1999). Failure of some populations to establish is common among invasive species because dispersers will not always

encounter favorable habitat during range expansion (Lockwood *et al.* 2005). However, these failures are unlikely to halt the spread of the invading species if established populations can still produce large numbers of dispersers (or propagules) to invade all of the available suitable habitat (propagule pressure) (Kolar and Lodge 2001; Lockwood *et al.* 2005).

3.4.4 Conclusions

The genetic diversity of the *Gambusia holbrooki* populations sampled in this study is low in comparison with home range estimates. The results of this study indicate that Queensland is the source of Tasmanian *Gambusia.* However, this could not be definitively confirmed and further research using more MSAT loci, more reference samples from Queensland and possibly analyzing mtDNA, is required to confirm this.

Of the core group of Tamar populations, TIWR is most likely to be driving the spread of *Gambusia* via propagule pressure (LN and CC did not persist, and W and R were discovered after TIWR). This finding is crucial in a management context because control and eradication efforts should focus on TIWR in an attempt to reduce the number of dispersers. However, it should also be noted that the modest natural range expansion reported here (Chapter 2) is insignificant compared to the spread that can and has been achieved via human assisted translocation, as evidenced by *Gambusia's* worldwide distribution. For this reason, public awareness campaigns are likely to be the most effective way of limiting further large scale spread of *Gambusia* in Tasmania.

3.5 References

Allendorf F. W. and Lundquist L. L. (2003) Population biology, evolution, and control of invasive species. *Conservation Biology* **17**, 24-30.

Arthington A. H. (1989) Diet of *Gambusia affinis holbrooki*, *Xiphophorus helleri*, *X. maculatus* and *Poecilia reticulata* (Pisces: Poeciliidae) in streams of southeastern Queensland, Australia. *Asian Fisheries Science* **2**, 193-212.

Arthington A. H. (1990) The role of habitat disturbance in the establishment of introduced warm-water fishes in Australia. In 'Introduced and translocated fishes and their ecological effects'. (Ed. DA Pollard) pp. 61-66. (Australian Government Publishing Service: Canberra Bureau of Rural resources proceedings).

Arthington A. H. and Lloyd L. N. (1989) Introduced Poeciliids in Australia and New Zealand. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 333-348. (Prentice Hall: New Jersey).

Baker S. C. and Clapham P. J. (2004) Modelling the past and future of whales and whaling. *Trends in Ecology and Evolution* **19**, 365-371.

Barrett S. C. H. and Kohn J. R. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In 'Genetics and conservation of rare plants'. (Eds DA Falk and KE Holsinger) pp. 3-30. (Oxford University press: New York).

Bonizzoni M., Guglielmino C. R., Smallridge C. J., Gomulski M., Malacrida A. R. and Gasperi G. (2004) On the origins of medfly invasion and expansion in Australia. *Molecular Ecology* **13**, 3845-3855.

Brown K. L. (1985) Demographic and genetic characteristics of dispersal in the mosquitofish, *Gambusia affinis* (Pisces: Poeciliidae). *Copeia* **3**, 597-612.

Brown K. L. (1987) Colonization by mosquitofish (*Gambusia affinis*) of a Great Plains river basin. *Copeia* **2**, 336-351.

Bryan M. B., Zalinski D., Filcek K. B., Libants S., Li W. and Scribner K. T. (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. *Molecular Ecology* **14**, 3757-3773.

Burns E. L., Eldridge M. D. B. and Houlden B. A. (2004) Microsatellite variation and population structure in a declining Australian Hylid *Litoria aurea*. *Molecular Ecology* **13**, 1745-1757.

Casterlin M. E. and Reynolds W. W. (1977) Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia* **55**, 125-127.

Clunie P., Stuart I., *et al.* (2002) 'A risk assessment of the impacts of pest species in the riverine environment in the Murray-Darling Basin.' Department of Natural Resources and Environment, Arthur Rylah Institute for environmental research, Project R2006, Heidelberg, Victoria.

Congdon B. C. (1992) The maintenance of genetic diversity in subdivided populations of the mosquitofish, *Gambusia holbrooki*. PhD thesis thesis, Griffith University.

Congdon B. C. (1994) Characteristics of dispersal in the eastern mosquitofish *Gambusia holbrooki*. *Journal of fish biology* **45**, 943-952.

Congdon B. C. (1995) Unidirectional geneflow and maintenance of genetic diversity in Mosquitofish *Gambusia holbrooki* (Teleostei: Poeciliidae). *Copeia* **1**, 162-172.

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Economidis P. S., Dimitriou E., Pagoni R., Michaloudi E. and Natsis L. (2000) Introduced and translocated fish species in the inland waters of Greece. *Fisheries Management and Ecology* **7**, 239-250.

Excoffier L., Smouse P. E. and Quattro J. M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application of human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Falush D., Stephens M. and Pritchard J. K. (2003) Inference of population structure: Extensions to linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.

Felsenstein J. (2006) 'PHYLIP Phylogeny Inference Package. (Ver. 3.66). (University of Washington: Seattle).

Garcia-Berthou E. (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* **55**, 135-147.

Garcia-Berthou E., Alcaraz C., Pou-Rovira Q., Zamora L., Coenders G. and Feo C. (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 453-463.

Gill H. S., Hambleton S. J. and Morgan D. L. (1999) Is the mosquitofish, *Gambusia holbrooki* (Poeciliidae), a major threat to the native freshwater species of south-western Australia? *Proceedings of the 5th Indo-Pacific Fish Conference*, 393-403.

Goodisman M. A. D., Matthews R. W. and Crozier R. H. (2001) Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia. *Molecular Ecology* **10**, 1423-1432.

Goudet J. (2001) 'FSTAT, a program to estimate and test gene diversities and fixation

indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Grapputo A., Bisazza A. and Pilastro A. (2006) Invasion success despite reduction of genetic diversity in the European populations of the eastern mosquitofish (*Gambusia holbrooki*). *Italian Journal of Zoology* **73**, 67-73.

Grapputo A., Boman S., Lindstrom L., Lyytinen A. and Mappes J. (2005) The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology* **2005**, 4207-4219.

Gutierrez-Estrada J. C., Prenda J., Oliva F. and Fernandez-Delgado C. (1998) Distribution and habitat preferences of the introduced mummichog *Fundulus heteroclitus* (Linneaus) in south-western Spain. *Estuarine, Coastal and Shelf Science* **46**, 827-835.

Hamer A. J., Lane S. J. and Mahony M. J. (2002) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* **132**, 445-452.

Haynes J. L. and Cashner R. C. (1995) Life history and population dynamics of the western mosquitofish: A comparison of natural and introduced populations. *Journal of fish biology* **46**, 1025-1041.

Hoffman J. I., Matson C. W., Amos W., Loughlin T. R. and Bickham J. W. (2006) Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Stellar's sea lion (*Eumetopias jubatus*). *Molecular Ecology* **15**, 2821-2832.

Howe E., Howe C., Lim R. and Burchett M. (1997) Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine & Freshwater Research* **48**, 425-434.

Hubbs C. (1999) Effect of Light Intensity on Brood Production of Livebearers *Gambusia* spp. *Transactions of the American Fisheries Society* **128**, 747-750.

Hughes J. M., Harrison D. A. and Arther J. M. (1991) Genetic variation at the *PGI* locus in the mosquitofish, *Gambusia affinis* (Poeciliidae), and possible effect on susceptability to an insecticide. *Biological Journal of the Linnean Society* **44**, 153-167.

Ivanstoff W. and Aarn (1999) Detection of predation on Australian native fishes by *Gambusia holbrooki*. *Marine and Freshwater Research* **50**, 467-468.

Kandl K. L. (2001) Effects of Inbreeding and Salinity Stress on Population Dynamics of Eastern Mosquitofish. *Transactions of the American Fisheries Society* **130**, 1224-1232.

Kats L. B. and Ferrer R. P. (2003) Alien predators and amphibian decline: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9**, 99-110.

Keane J. P. (2002) Population structure, reproductive onset and temperature tolerance of the mosquitofish *Gambusia holbrooki* in northern Tasmania. Australian Maritime College.

Keane J. P. and Neira F. J. (2004) First record of mosquitofish, *Gambusia holbrooki*, in Tasmania: stock structure and reproductive biology. *New Zealand Journal of Marine and Freshwater Research* **38**, 857-868.

Kennedy P. K., Kennedy M. L., Zimmerman E. G., Chesser R. K. and Smith M. H. (1986) Biochemical genetics of mosquitofish. V. Perturbation effects on genetic organisation of populations. *Copeia* **1986**, 937-945.

Kolar C. S. and Lodge D. M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* **16**, 199-204.

Komak S. and Crossland R. (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* **27**, 185-189.

Lande R. and Barrowclough F. (1987) Effective population size, genetic variation, and their use in population management. In 'Viable populations for conservation'. (Ed. ME Soule) pp. 87-123. (Cambridge University Press: Cambridge).

Lasota R., Hummel H. and Wolowicz M. (2004) Genetic diversity of European populations of the invasive soft-shell clam, *Mya arenaria* (Bivalvia). *Journal of the Marine Biological Association of the United Kingdom* **84**, 1051-1056.

Lawler S. P., Dritz D., Strange T. and Holyoak M. (1999) Effects of Introduced Mosquitofish and Bullfrogs on the Threatened California Red-Legged Frog. *Conservation Biology* **13**, 613-622.

Leberg P. L. (2002) Estimating allelic richness: Effects of sample size and bottlenecks. *Molecular Ecology* **11**, 2445-2449.

Lindholm A. K., Breden F., Alexander H. J., Chan W., -K., Thakurta S. G. and Brooks R. (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Molecular Ecology* **14**, 3671-3682.

Lloyd L. (1990a) Ecological interactions of *Gambusia holbrooki* with Australian native fish. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 94-97. (Austalian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. (1990b) Native fishes as alternative to the exotic fish, *Gambusia*, for insect control. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 115-122. (Australian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. N., Arthington A. H. and Milton D. A. (1986) The mosquitofish - a valuable mosquito control agent or a pest? In 'The ecology of exotic plants and animals: some Australian case studies'. (Ed. Kitching). (John Wiley and Sons: Brisbane).

Lockwood J. L., Cassey P. and Blackburn T. (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* **20**, 223-228.

Meffe G. K. (1984) Effects of abiotic disturbance in coexistence of of predator-prey fish species. *Ecology* **65**, 1525-1534.

Milton D. A. and Arthington A. H. (1983) Reproductive biology of *Gambusia affinis holbrooki* (Baird and Girard), *Xiphophorus helleri* (Gunther) and *X. maculatus* (Heckel) (Pisces; Poeciliidae) in Queensland, Australia. *Journal of Fish Biology* **23**, 23-41.

Morgan D. L., Gill H. S., Maddern M. K. and Beatty S. J. (2004) Distribution and impacts of introduced freshwater fishes in Western Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 511-523.

Nei M. (1972) Genetic distance between populations. *American Naturalist* **106**, 283-292.

Oosterhout C. V., Hutchinson W. F., Wills D. P. M. and Shipley P. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Paetkau D., Slade R., Bryden M. and Estoup A. (2004) Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. *Ecology* **13**, 55-65.

Peakall R. and Smouse P. E. (2005) 'GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research.'pgs. (The Australian National University. http://www.anu.edu.au/bozo/GenAlEx: Canberra, Australia).

Pemberton M., Slate J., Bancroft D. R. and Barrett J. A. (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage or population studies. *Molecular Ecology* **4**, 249-252.

Pen L. J., Potter I. C. and Calver M. C. (1993) Comparisons of the food niches of three native and two introduced fish species in an Australian river. *Environmental biology of fishes.The Hague* **36**, 167-182.

Piry S., Alapetite A., Cornuet J.-M., Paetkau D., Baudouin L. and Estoup A. (2004) GeneClass2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**, 536-539.

Pritchard J. K., Stephens M. and Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-949.

Pritchard J. K., Wen X. and Falush D. (2007) 'Documentation for STRUCTURE software:

Version 2.2. (Ver. http://pritch.bsd.uchicago.edu/software). (Department of Human Genetics: University of Chicago).

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* **15**, 339-365.

Rannala B. and Mountain J. L. (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Science, USA* **94**, 9197-9201.

Raymond M. and Rousset F. (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Rice W. R. (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Rivas L. R. (1963) Subgenera and species groups in the poeciliid fish genus *Gambusia* Poey. *Copeia* **1963**, 331-347.

Rollins L. A., Woolnough A. P. and Sherwin W. B. (2006) Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Sakai A. K., Allendorf F. W., *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**, 305-332.

Schussman H., Geiger E., Mau-Crimmins T. and Ward J. (2006) Spread and current potential distribution of an alien grass, *Eragrostis lehmanniana* Nees, in the southwestern USA: comparing historical data and ecological niche models. *Diversity and Distributions* **12**, 582-592.

Shaw P. W., Pierce G. J. and Boyle P. R. (1999) Subtle population structuring within a highly vagile marine invertebrate, the veined squid, *Loligo forbesi*, demonstrated with microsatellite DNA markers. *Molecular Ecology* **8**, 407-417.

Smith M. H., Scribner K. T., Hernandez J. D. and Wooten M. C. (1989) Demographic, spatial and temporal genetic variation in *Gambusia*. In 'Ecology and evolution of livebearing fishes (Poeciliidae)'. (Eds GK Meffe and FF Snelson) pp. 235-257. (Prentice Hall: Englewood Cliffs, NJ).

Spencer C. C., Chlan C. A., Neigel J. E., Scribner K. T., Wooten M. C. and Leberg P. L. (1999) Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis*. *Molecular Ecology* **8**, 157-168.

Truett G. E., Heeger P., Mynatt R. L., Truett A. A., Walker J. A. and Warman M. L. (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques* **29**, 52-54.

Tsutsui N. D., Suarez A. V., Holway D. A. and Case T. J. (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Science, USA* **97**, 5948-5953.

Vrijenhoek R. C. (1979) Genetics of sexually reproducing fish in a highly fluctuating environment. *American Naturalist* **113**, 17-29.

Ward D. L., Schultz A. A. and Matson P. G. (2003) Differences in swimming ability and behavior in response to high water velocities among native and nonnative fishes. *Environmental Biology of Fishes* **68**, 87-92.

Wilson F. (1960) 'A review of the biological control of insects and weeds in Australia and Australian New Guinea.' 102 pgs. (Commonwealth Agricultural Bureaux: Bucks, England).

Wooten M. C. and Lydeard C. (1990) Allozyme variation in natural contact zone between *Gambusia affinis* and *Gambusia holbrooki*. *Biochemical Systematics and Ecology* **18**, 169-173.

Wooten M. C., Scribner K. T. and Smith M. H. (1988) Genetic variability and systematics of *Gambusia* in the southeastern United States. *Copeia* **2**, 283-289.

Zane L., Nelson W. S., Jones A. G. and Avise J. C. (1999) Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* **12**, 61-69.

CHAPTER 4:

The effects of attempted eradication on the genetic diversity of two populations of *Gambusia holbrooki*

4.1 Introduction

Mosquitofish, *Gambusia holbrooki* (Girard) and *Gambusia affinis* (Baird & Girard), are small live-bearing, ovoviviparous fish, native to Central America and southern North America (Rivas 1963; Wooten *et al.* 1988). Mosquitofish are one of the most widely distributed freshwater fishes in the world due to their introduction into many countries for the purpose of mosquito control (Wilson 1960; Courtenay and Meffe 1989; Lloyd 1990a). Unfortunately, *Gambusia* spp. have also had negative impacts on many native species and habitats where they have been introduced (Arthington and Lloyd 1989; Lloyd 1990b; Webb and Joss 1997), and are considered a pest species outside their native range. These impacts include competition and direct predation on native fish (Howe *et al.* 1997; Ivanstoff and Aarn 1999) and frogs (Hamer *et al.* 2002; Kats and Ferrer 2003), and promotion of algal blooms caused by zooplankton predation (Hurlbert and Mulla 1981).

It is widely accepted that exotic species are virtually impossible to eradicate if they are allowed sufficient time to acclimatize to local conditions and establish stable reproducing populations (Crooks and Soule 1999; Myers *et al.* 2000; Bax and McEnnulty 2001). If complete eradication is logistically unachievable, it may then be possible to restrict or slow the spread of some species by selectively targeting source populations within an identified source-sink dynamic (Pulliam 1988; Hanski 1999). In a heterogenous environment, populations may exist as a metapopulation where a number of populations occupy discrete habitat patches and are connected by dispersal (Hanski, 1999), as is the case with *Gambusia* in Tasmania (*refer to* Chapters 2 and 3). In a metapopulation, some populations may act as a source (where total emigration exceeds immigration) and others as a sink (which depend on immigration to persist) (Pulliam 1988; Hanski 1999).

A small number of *G. holbrooki* were illegally introduced into a private dam in Legana, northern Tasmania, in 1992 (Tasmanian Inland Fisheries Service (IFS hereafter) *unpublished data*). The fish were thought to be restricted to two small private dams in Legana, but in 2000 they were re-discovered 8 km from the original introduction site in the Tamar Island Wetland Reserve (Keane and Neira, 2004). Since that time, yearly distribution surveys have discovered populations at a number of sites within an 18 km stretch of the upper Tamar Estuary (Chapter 2). Since their introduction in the early 1990's, the IFS attempted to eradicate or control the fish in small enclosed water bodies wherever possible (*see* Table 4.1; IFS *unpublished data*). Populations in discrete water bodies can act as source populations for natural or human assisted dispersal but are considered to be at low risk of re-infestation due to physical barriers to fish dispersal (except during flood events).

In this study, eradication of *Gambusia* was attempted from two small dams near suburban Launceston (Tasmania, Australia) in an effort to control the spread of the fish. Both dams supported large *Gambusia* populations that provided an ideal refuge for the fish to establish reproducing populations and facilitate range expansion by dispersal. The eradication attempt offered a unique opportunity to evaluate the effectiveness of the methods employed, to document any recovery of the *Gambusia* populations, and to assess the impact of attempted eradication on *Gambusia* population genetic diversity. Information regarding population recovery times and subsequent effect on population genetic diversity will provide valuable information for future management of this invasive fish.

Table 4.1. Summary of habitat characteristics, materials and procedures used in eradications of *Gambusia holbrooki* in Tasmania. *Note that Legana dam 2 is the site "Legana dam" in the present study. Table was compiled from Inland Fisheries Service records.

Site Location	Description	Size	Date	Toxicant used	Methods	Successful?
Legana Dam 1	Small farm dam with edges and centre heavily vegetated with bullrushes	15 x 6 m dia. and 1.5m depth	Mid 1993	Fenitrothion or Cypermethrin	Detailed records were not kept, however the dam and downstream drain were also treated.	**No**. Post treatment monitoring occurred after 3 months. No fish found in periodic checks up until 1998
			March 2002	Limil ($Ca(OH)_2$)	225 kg of Limil applied and mixed using a 3m inflatable boat with 8 hp motor	**Yes**. Yearly monitoring has been unable to detect any *Gambusia*
*Legana Dam 2	Small farm dam consisting of two connected ponds. Dense cover of macrophytes and reeds around the edge	40 x 20 m dia. and 1 m deep	March 2002	Limil ($Ca(OH)_2$)	375 kg of Limil applied and mixed using a 3m inflatable boat with 8 hp motor	**No**. *Gambusia* re-established by April 2003
			May 2003	Limil ($Ca(OH)_2$)	575 kg of Limil applied and mixed using a 3m inflatable boat with 8 hp motor	**No**. Heavy rain prior to treatment significantly increased the amount of water to be treated
Snug Dam	Farm dam isolated from other water storages. Accessible by general public and dense macrophyte coverage with many habitat refuges for fish.	15 x15m dia. and 0.5m deep	Jul 2003	Limil ($Ca(OH)_2$)	240kg of Limil applied and mixed using a 3m inflatable boat with 8 hp motor	**No**, live fish found after several months. Too many refuges for fish in macrophytes.
			Oct 2003	Rotenone	Rotenone was applied with a 500L spray unit at a conc. of 150ug/L. Dam volume was reduced by 80% prior to rotenone application by siphoning water out through a filter. Trout introduced after 2 weeks suffered no ill effects from the piscicide.	**Yes**. No *Gambusia* found in Apr 2005
Kingston Dam	Small farm dam. *Gambusia* population result of transfer of aquatic plants from Snug.	20 x15m dia. and 2m deep	Nov 2003	Limil ($Ca(OH)_2$)	600kg of Limil was applied and mixed using a 3m inflatable boat with 3 hp motor. Edges were treated with hand spreading	**Yes**. No *Gambusia* found in Apr 2005

4.2 Materials and methods

The two eradication sites, Riverside and Legana dams, are located near the upper reaches of the Tamar Estuary, in northern Tasmania (Fig. 4.1). The Tamar Estuary is a drowned river valley influenced by semi diurnal tides that extend to St. Leonards on the North Esk River and first basin on the South Esk (Foster *et al.* 1986). Tidal patterns and river flows from the North and South Esk rivers control the distribution of salinity and its structure throughout the estuary (Foster *et al.* 1986).

4.2.1 Site descriptions

4.2.1.1 Riverside Dam

Riverside dam is a farm dam between the suburbs of Riverside and Legana, north of Launceston city (41° 22" 683' S: 147° 04" 173' E). The dam is relatively large (2160 m^2, 206m circumference) and shallow, with a central island (Fig. 4.2). Prior to eradication of *Gambusia,* water was free of suspended matter, with a high percentage cover of submerged filamentous macrophytes and emergent reeds. The water in Riverside dam at the time of eradication was slightly saline as it is tidally connected to the Tamar Estuary via a concrete pipe. It is believed that this is how *Gambusia* colonised the dam. Prior to water extraction, a concrete plug was inserted and sealed into the pipe at low tide to permanently block fish and water movement between the dam and the estuary. Prior to and during the eradication, seven species of fish were encountered (including *Gambusia holbrooki)* at Riverside dam. These were tench (*Tinca tinca),* short-finned eel (*Anguilla australis),* common jollytail

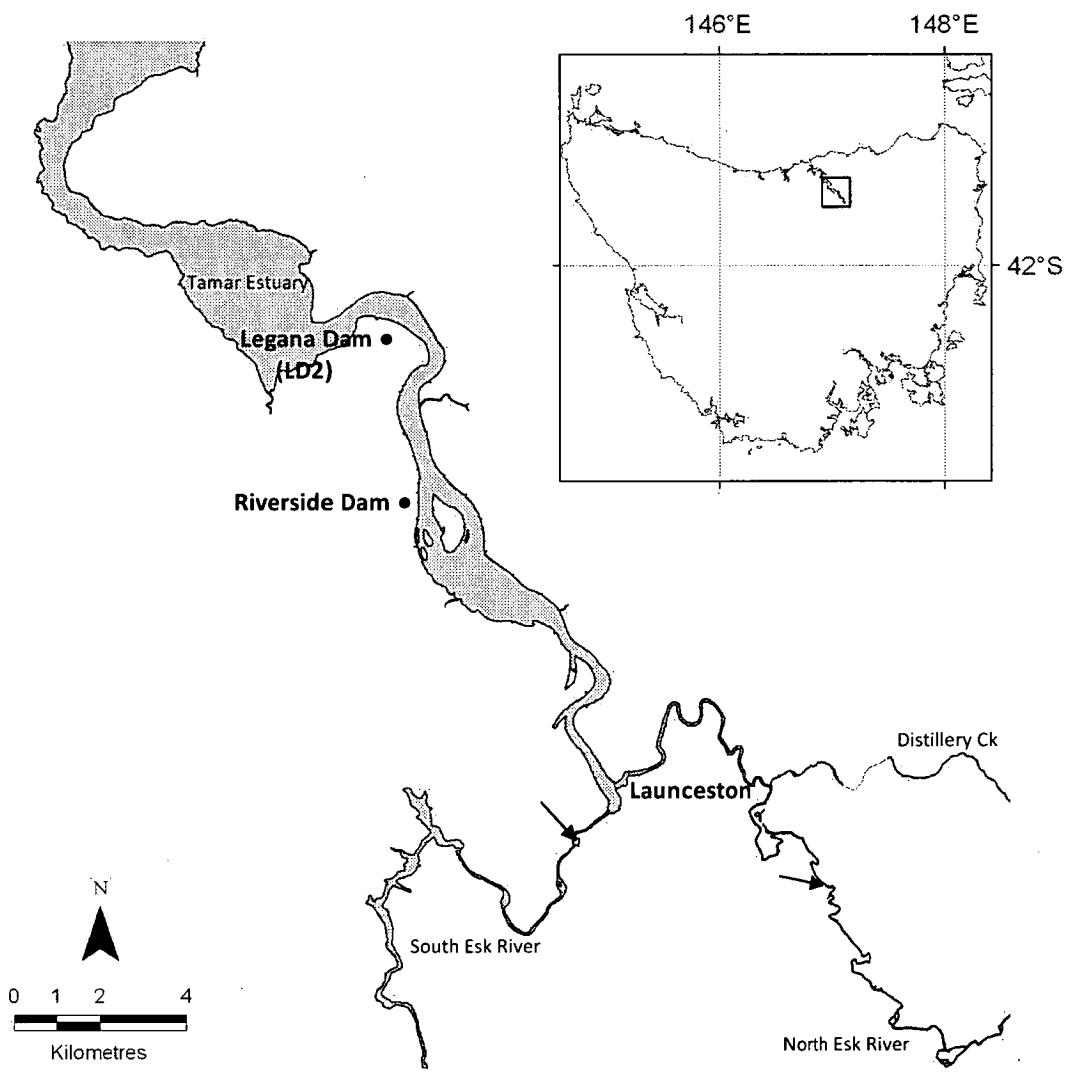

Figure 4.1. Map of the upper reaches of the Tamar Estuary (northern Tasmania) showing the location of the study sites Riverside and Legana Dams (LD2). Arrows indicate approximate extent of tidal influence.

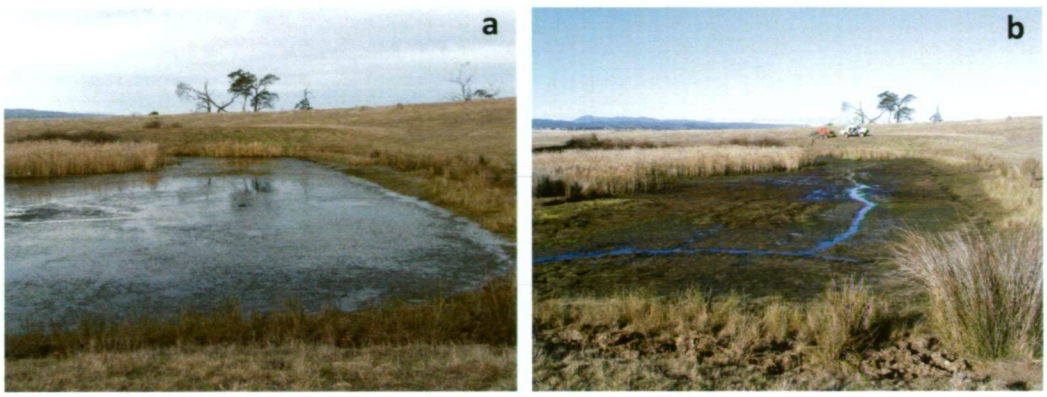

Figure 4.2. Riverside Dam, a) before draining, b) after pumping was completed prior to treatment with lime.

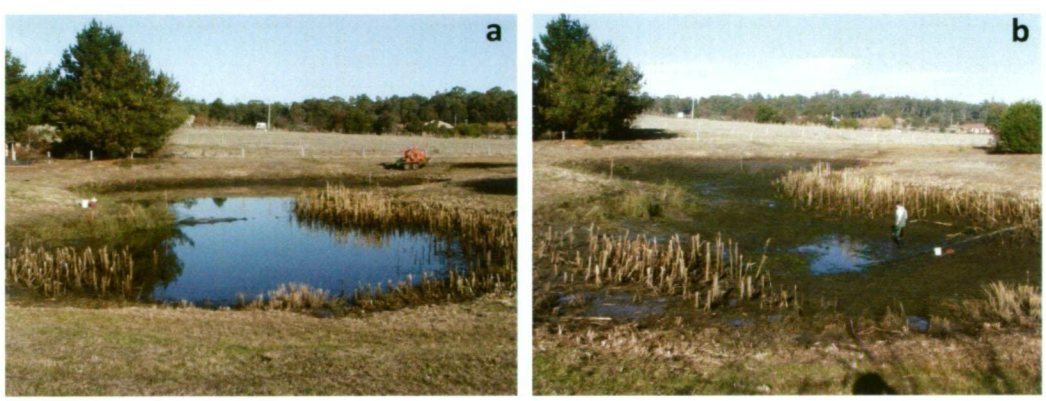

Figure 4.3. Legana Dam LD2, (a) before draining, (b) after pumping was completed, prior to treatment with lime.

(*Galaxias maculatus),* flathead gudgeon (*Philypnodon grandiceps),* small-mouthed hardyhead (*Atherinosoma microstoma)* and freshwater flathead (*Pseudaphritis urvillii).* Large populations (>100 individuals) of all species were encountered.

4.2.1.2 Legana Dam

Legana dam (LD2) is situated in the rural suburb of Legana, north of the Launceston metropolitan area (41° 20" 580' S: 147° 03" 791' E). LD2 is a relatively small enclosed water body (1048 m^2, 135 m circumference) consisting of two connected ponds (one deeper than the other) (Fig. 4.3). The dam has a muddy substrate covered with a dense mat of macrophytes and emergent reeds encircling the edge. Prior to eradication, LD2 supported a large population of *Gambusia* (> 60 fish 0.5 m^{-2} in summer) and its slightly turbid water was entirely fresh. The resident *Gambusia* population was illegally introduced by the prior property owners during the early 1990's (IFS *unpublished data*). Although there is no tidal connection to the Tamar Estuary, at high water level it is possible for the dam to overflow and allow fish to move into the estuary. Eradication of *Gambusia* has been attempted at this site on two occasions prior to the current study (Table 4.1). A total of five fish species were recorded at LD2 (including *G. holbrooki*) prior to eradication; namely short-finned eel (*Anguilla australis),* common jollytail (*Galaxias maculatus),* spotted minnow (*Galaxias truttaceus)* and brown trout (*Salmo trutta).* The *Galaxias* species were present in very small numbers (1 or 2 individuals) and the brown trout had been introduced several months prior to the eradication attempt to ascertain whether they would feed on *Gambusia.*

4.2.2 Eradication methods

Eradications at both dams took place in late May 2005. Unseasonally low rainfall had resulted in low water levels in both dams, making it ideal to attempt the eradication. Hydrated lime (slaked lime, calcium hydroxide $Ca(OH)_2$) was the toxicant chosen to eradicate *Gambusia* at both sites because it is more effective than rotenone at low temperature and does not have such an adverse effect on the macro-invertebrate community (Morrison 1977; Clair and Hindar 2005). A four inch Sterling SPP100 pump (25 $Lsec^{-1}$ max. rate) was used to pump out water from both dams. At Riverside dam, as the water approached base levels, channels were dug and the pumping rate (r.p.m.) was reduced to remove as much water as possible. The pump's outlet hose was run through a heavy duty 1mm mesh stainless steel screen in order to prevent *Gambusia* escaping. Lime was applied by hand and extra lime added to deeper areas. A total of 180 kg of lime was used for the initial treatment.

A similar process was used at LD2. Water pumping was quicker due to the dams' smaller overall surface area (1048 m^{-2}) and the steep sides that confined the remaining water to a smaller area (Fig. 4.3b). A total of 130 kg of lime was spread by hand at LD2 after water extraction.

In the days and months following initial lime treatment both dams were monitored continually for signs of fish survival. If live fish were observed, further lime treatments were undertaken to ensure that no *Gambusia* had survived. A total of 290 kg and 150 kg of lime was used at Riverside and LD2 respectively.

4.2.3 Gambusia abundance monitoring

Monthly abundance sampling of *Gambusia* was carried out between April 2004 and July 2006 at LD2. Fish were sampled using a 0.5 m^{-2} aluminum throw trap. On each sampling occasion a stratified sampling design was employed. The perimeter of the dam was divided into 10 m transects that were randomly selected to deploy the throw trap. Fish were sampled with at least 20 replicate trap throws on each sampling occasion. All fish were euthanized using MS-222 and retained for subsequent enumeration. Abundance estimates were obtained at LD2 for at least 12 months before and after eradication was attempted. *Gambusia* abundance data was not obtained at Riverside dam due to the relatively recent discovery of *Gambusia* at the site. General observations of *Gambusia* population abundance were made both before and after the eradication attempt at Riverside dam.

4.2.4 Genetic sample collection

Approximately 50 *G. holbrooki* were collected immediately before the eradication attempt and when post-eradication populations were discovered that were sufficient for population studies (November 2005 for Riverside and March 2006 for Legana). All fish were collected using a fine mesh dip-net, and were stored in 70-95% ethanol immediately after capture.

4.2.5 DNA preparation, PCR amplification and microsatellite genotyping

DNA was isolated from small caudal fin clips taken from ethanol preserved specimens. DNA was extracted using a simple method involving a 35 minute incubation in lysis solution followed by the addition of neutralizing solution and storage at -20°C (Truett *et al.* 2000). DNA extracts were stored at -20°C until just prior to use in PCR reactions. Six microsatellite loci (Gafμ2, Gafμ4 & Gafμ7 and M*f*1, M*f*6 & M*f*13) were selected for assaying diversity in pre- and post-eradication populations of *Gambusia* (Spencer *et al.* 1999; Zane *et al.* 1999). Amplification of Gafμ2, Gafμ4, & Gafμ7 were performed in a 15 μl final reaction volume containing 5 μl of genomic DNA extract, 0.6 pmol of end-labeled forward primer and unlabelled reverse primer, 100 μM of each dNTP, 1.5 mM $MgCl_2$, 0.6 U of BIOTAQ™ DNA polymerase (Bioline, UK), and 1.5μl of 10x PCR buffer (consisting of 160 mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20). PCR amplification and cycling for loci M*f*1, M*f*6 and M*f*13 followed the conditions of (Zane *et al.* 1999). All microsatellite loci were amplified using an Eppendorf gradient mastercycler (Eppendorf, Germany). The cycling conditions for all Gafμ loci followed the protocol described by (Spencer *et al.* 1999). Fluorescently labeled PCR products were separated by size and analysed using the Beckman Coulter software package, CEQ 8000™ genetic analysis system, version 8.0. Genotypes were assigned to each individual fish based on the size of the PCR products (base pairs) observed in the electropherogram relative to a size standard. A total of 53 (pre-eradication) and 48 (post-eradication) fish were genotyped at LD2, and 49 (pre-eradication) and 48 (post-eradication) fish at Riverside dam.

4.2.6 Statistical analysis

A univariate two way ANOVA was used to compare the monthly density of *Gambusia* (pre- and post-eradication) at Legana. Homogeneity of variances was determined by examining residual plots and data were $\log_{10}x+1$ transformed when this assumption was violated. A one way ANOVA with planned contrasts was used to compare corresponding months (pre- and post-eradication).

Observed (H_o) and expected (H_e) heterozygosities were derived using the software package Genetic Data Analysis (or GDA) (Lewis and Zaykin 2001). Fixation index (F_{IS}), Allele frequencies and Allelic diversity (A) were calculated for each locus/population using the genetic data analysis program GenAlEx ver. 6.0 (Peakall and Smouse 2005). Chi-square analyses were used to test for differences in allele frequencies between pre- and post-eradication populations at both sites. To evaluate changes in effective population size (N_e) and temporal change in allele frequencies (F_k), the approach outlined by (Waples 1989) was applied using the software package, NeEstimator (Peel *et al.* 2004). Confidence intervals for F_k were calculated following Plan II, equation #16 of (Waples 1989).

To compare pre- and post-eradication Allelic diversity (A), a single factor ANOVA was utilized. The non-parametric Mann-Whitney U test was used to test for significant differences between observed levels of heterozygosity (H_o) in pre-and post-eradication populations at both locations. Deviation from Hardy-Weinberg expectations was assessed using the exact test disequilibrium analysis in GDA (shufflings set at 10,000). Evidence of genotyping errors in the data set were assessed using the program MICRO-CHECKER (Oosterhout *et al.* 2004). The significance level for all analyses was $\alpha = 0.05$. All statistical

analyses were carried out using the statistical software package SPSS (ver 14.0 for windows, SPSS Inc.), unless otherwise stated.

4.3 Results

Riverside dam was completely drained after 17 hours of pumping. Based on the pump's specifications and the r.p.m., an estimated volume of 1.098 Megalitres of water was removed from Riverside dam. After initial lime addition the pH increased from 7.3 to 12.7 within half an hour. The response to liming was immediate with fish breaking the surface and beginning to die within minutes of the first application. At LD2, no fish were observed at the dam sides during draining and all descended to the bottom of the two main pools. The dam was completely drained with 9 hours of pumping and approximately 0.702 Megalitres (or 702 kL) of water was removed. The pH rose from 8.7 to 13.2 within half an hour of lime addition. At first, fish appeared unaffected by the lime, but after approximately ten minutes they began to die and float on the water surface.

4.3.1 Effect of eradication on population dynamics

Eradication of *Gambusia* at both sites was unsuccessful. Several live *Gambusia* were captured at Riverside dam 17 days after the initial lime treatment. The eradication initially appeared successful at LD2 however after eight months a single fish was sighted in January 2006, and by nine months post-eradication (Feb 2006) fish densities were sufficient to be detected in monthly density surveys (Fig. 4.4). The *Gambusia* population at LD2 did not recover to pre-eradication levels over the 2005/2006 summer and estimates of post-eradication density were significantly lower than pre-eradication densities ($F = 15.21$, $df = 5, 228$ $P < 0.001$). Fish density was significantly lower post-eradication in all monthly comparisons (all planned contrasts, $P < 0.001$).

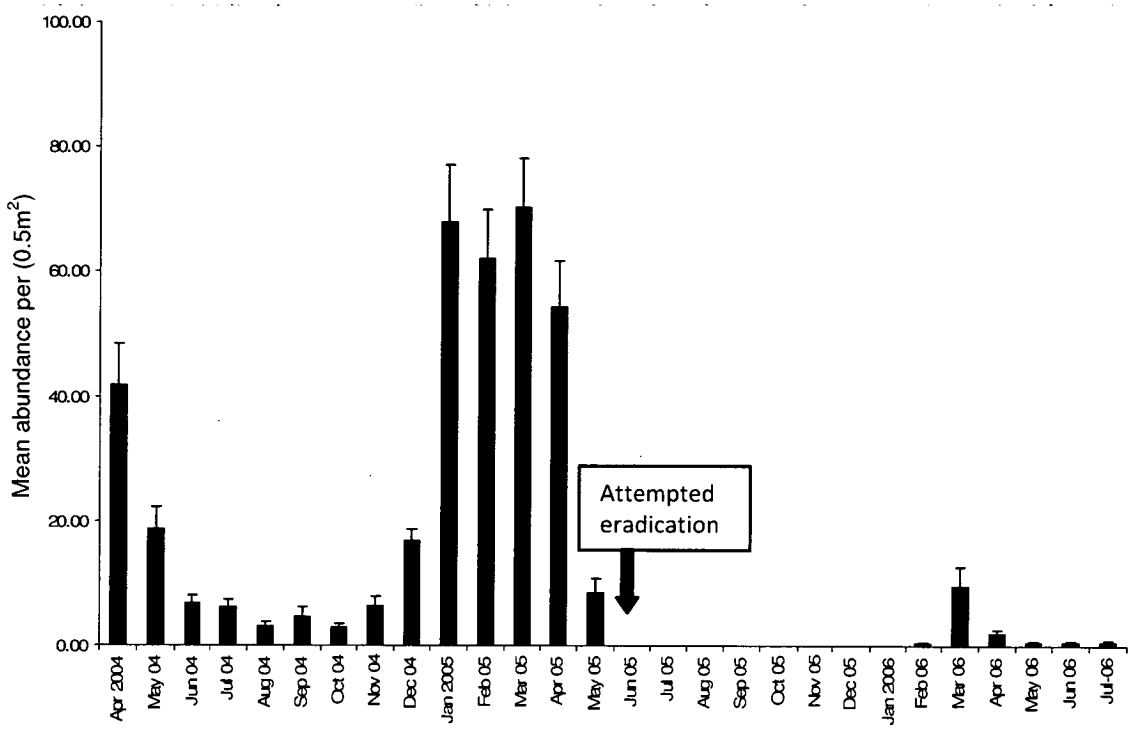

Figure 4.4. Mean abundance of *G. holbrooki* at Legana dam (LD2). Error bars represent standard errors.

4.3.2 Genetic consequences of eradication

Two of the loci, M*f*1 and M*f*6, were monomorphic in both populations and excluded from further analysis. The allele frequencies of pre- and post-eradication populations were significantly different at both sites (Riverside – Chi square, $df = 10$, $P < 0.001$; LD2 – Chi square, $df = 8$, $P < 0.001$). The population bottleneck induced by the eradication attempt produced more obvious changes in allele frequency patterns at LD2 compared to Riverside (Fig. 4.5). Two alleles were absent in the post-eradication population of *Gambusia* at LD2 (allele 162 at loci M*f*13 and 155 at Gafμ2). No alleles were lost at any of the loci examined at Riverside, however the frequencies of alleles at loci M*f*13 and Gafμ4 were different (Fig. 4.5). The rare alleles at Gafμ4 (262 and 266) were more common after the eradication (Fig. 4.5). The temporal estimate of allele frequency change (F_k) was lower at Riverside [0.111 (95% CI=0.058-0.319)] than at LD2 [0.422 (95% CI=0.192-1.545)]. Estimates of effective population size (N_e) indicate that less than ten individuals survived at Riverside [6 (95% CI=2-14)] and less than five at LD2 [1 (95% CI=0-3)] after the eradication attempt.

Mean allelic diversity (A) of the Riverside population was not reduced by the eradication attempt (Table 4.2). At LD2 there was a slight reduction in A from 2.25 to 1.75 (Table 4.2). However, this loss of allelic diversity was not statistically significant ($F = 2$, df = 1, 6, $P = 0.207$). There was no significant change in observed heterozygosity (H_o) in either population as a result of the eradication attempt (Mann-Whitney U, both $P > 0.05$) (Table 4.2). Fixation index (F_{IS}) changed slightly in both populations after the eradication attempt. There was slightly more inbreeding at Riverside compared to LD2 (Table 4.2). However, across all populations F_{IS} was close to zero (0.086) indicating very

little inbreeding.

There were three significant deviations from Hardy-Weinberg expectations, all in pre-eradication populations of *Gambusia* (two in Riverside and one in LD2) (Table 4.3). The most severe and statistically significant deviation occurred at Gafμ4 pre-eradication at Riverside where MICROCHECKER predicts a possible null allele is present (Table 4.3).

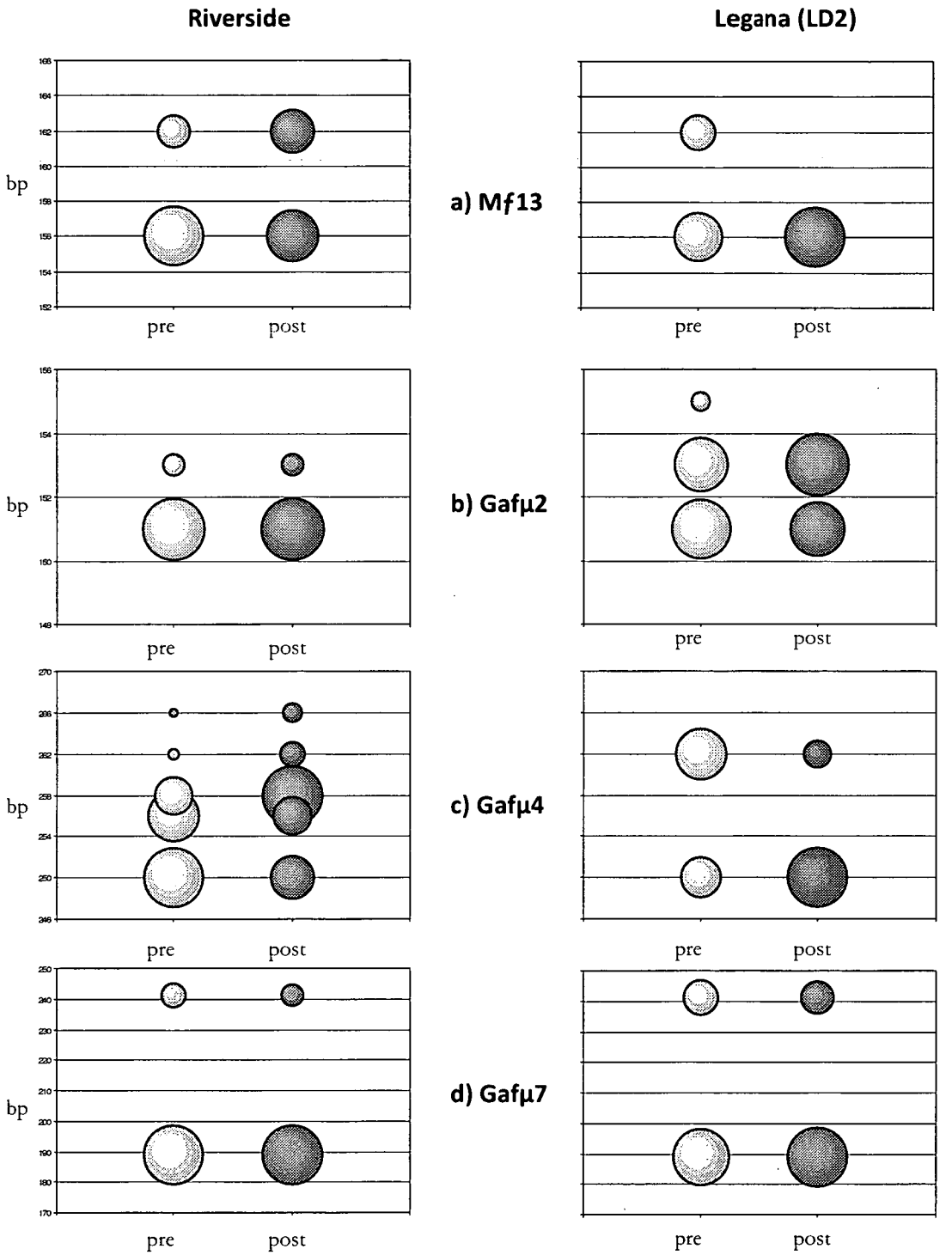

Figure 4.5. Allele frequencies of *Gambusia holbrooki* populations at Riverside and Legana dams at four microsatellite loci a) M*f*13, b) Gafμ2, c) Gafμ4, d) Gafμ7. Pre-eradication allele frequencies are displayed in white and post-eradication are in grey. The areas correspond to the frequencies of the respective alleles at each locus.

Table 4.2. A summary of the genetic variation at four microsatellite loci in two *Gambusia holbrooki* populations. **N,** the mean sample size; ***A,*** the mean number of alleles per locus; $\mathbf{H_o}$, mean direct count heterozygosity; $\mathbf{H_e}$, the mean Hardy-Weinberg expected heterozygosity; $\mathbf{F_{IS}}$ the inbreeding coefficient. Standard errors in parentheses.

Population	N	*A*	H_o	H_e	F_{IS}
Riverside					
Pre-eradication	48.50 (0.05)	2.75 (0.75)	0.272 (0.028)	0.357 (0.101)	0.247
Post-eradication	48 (0)	2.75 (0.75)	0.364 (0.096)	0.407 (0.117)	0.116
Legana (LD2)					
Pre-eradication	50.75 (0.47)	2.25 (0.25)	0.452 (0.061)	0.462 (0.033)	0.032
Post-eradication	47.75 (0.25)	1.75 (0.25)	0.299 (0.108)	0.284 (0.103)	-0.07

Table 4.3. Hardy-Weinberg expectation deviations at four microsatellite loci in pre- and post-eradication populations of *Gambusia holbrooki*. Significant probability values are represented in bold. * represents possible null allele.

Population	Mf13	Gafμ2	Gafμ4	Gafμ7
Riverside				
Pre-eradication	**0.014**	0.829	***0.000**	0.668
Post-eradication	0.227	0.271	0.055	0.286
Legana (LD2)				
Pre-eradication	0.872	0.222	0.274	**0.018**
Post-eradication	monomorphic	0.885	0.459	0.931

4.4 Discussion

4.4.1 Eradication failure and population recovery

The ultimate failure of eradication attempts at both sites can be attributed to a number of factors. Riverside dam has a large surface area and a complex shallow structure. As the water level dropped during pumping, many small refuges were created naturally and by footprints in the mud. Over time, water seemed to fill in or seep back into the pools that had been drained, providing a refuge for any surviving fish. The situation was similar at LD2 although not as pronounced. Heavy rainfall several days after the initial lime application (50mm from 1-17 June) also caused the lime to be quickly diluted and the pH rapidly decreased, thereby increasing the chance of survival of remaining *Gambusia.*

This study reinforces that total eradication of unwanted species is often extremely difficult, expensive, time consuming, and can require the use of extreme methods to achieve success (Ehler 1998; Myers *et al.* 2000; McEnnulty *et al.* 2001). The only way to ensure complete eradication of *Gambusia* from both sites would be to completely drain and dry out the habitat for several months after the addition of a toxicant, so that any fish that survive the initial application of toxicant are unable to find refuge and survive to found the next generation.

Given *Gambusia*'s high reproductive potential (Robbins *et al.* 1987; Leberg 1992; Zane *et al.* 1999; Koya and Kamiya 2000; Keane and Neira 2004), it is not surprising that both populations recovered quickly from the eradication attempt. However, there were differences in the rate of recovery of the two populations. The Riverside population

recovered faster than LD2, with survivors being observed soon after the eradication attempt, indicating that the number of survivors was much lower at LD2. The estimates of effective population size calculated for the present study also support this as N_e was greater at Riverside because the temporal change in allele frequencies was smaller than at LD2.

Given that fish were observed a short time after the eradication attempt at Riverside, and there was sufficient time for 2-3 generations over the following summer, the *Gambusia* population at Riverside would likely be at pre-eradication size by March 2006. As other fish species were also negatively affected by the eradication, the recovery of the *Gambusia* population may also be enhanced by the reduction in the number of potential competitors. Although re-colonization of the dam by *Gambusia* (from outside) has been prevented by blocking the drain to the estuary, no other fish species are likely to have been able to enter and compete with *Gambusia*. The drain blockage also stopped water exchange with the estuary and the dam is now entirely freshwater post-eradication. These factors increase the suitability of the habitat for *Gambusia* and the eradication attempt may ultimately lead to a larger population at the site.

The recovery of the population at LD2, although slower than at Riverside, will probably only require one further summer breeding season (Nov 06-Apr 07) to reach pre-eradication levels based on abundance data collected before the eradication. Population growth will be unimpeded by competition with other species and intra-specific competition should be low because the population will be well below carrying capacity.

4.4.2 Genetic consequences of eradication attempt

If a population is able to survive an eradication attempt it will have survived a severe temporary reduction in size, or "bottleneck" (Nei *et al.* 1975). Bottlenecks can influence the distribution of genetic variation within and among populations and can lead to loss of genetic diversity, inbreeding, and may reduce the potential of a population to respond to selective pressures (Nei *et al.* 1975). The severity of the bottleneck and the time taken for the population to reach pre-bottleneck size also influences the effect on the population diversity (Nei *et al.* 1975). Bottlenecks have been studied extensively using *Gambusia* and other organisms as models (Nei *et al.* 1975; Leberg 1992; Spencer *et al.* 2000; Leberg 2002). Severe bottlenecks in experimental *Gambusia* populations result in a reduction of allelic diversity in the new population compared to the source (Spencer *et al.* 2000), but populations recover quickly and exhibit high rates of population growth until the carrying capacity of the small experimental ponds is reached (2-3 generations) (Spencer *et al.* 2000).

The life history of *Gambusia* spp. enables them to be particularly resistant to the negative effects of genetic bottlenecks (Brown 1987). Firstly, they are a short-lived species with enormous reproductive potential, making them capable of rapid population increase following a bottleneck event (Leberg 1992). Multiple insemination of females maximizes genetic mixing within a population and females are able to store sperm from one breeding season to the next, enabling a single individual to establish a new population (Zane *et al.* 1999). In addition, factors such as *Gambusia*'s broad dietary (Chapter 2) and physiological requirements (Pyke 2005), and ability to outcompete other species in disturbed habitats (Arthington 1990) would also contribute to their rapid recovery from

bottleneck events.

We would expect to see a reduction in allelic diversity if the eradication attempt resulted in a severe population bottleneck (Nei *et al.* 1975). The magnitude of diversity loss will be dependent on the severity of the bottleneck (Nei *et al.* 1975). One of the best and most reliable indicators of whether a population has undergone a bottleneck is a loss of Allelic diversity (A) (Spencer *et al.* 2000). In this study, there was no loss of A at Riverside, while at LD2 there was a small (but not significant) reduction. This reinforces the fact that the Riverside population did not experience as severe a bottleneck as LD2. However, there may have been a loss of diversity that was unable to be detected with the markers used in this study. It was unfortunate that two of the loci used in this study were invariant and if individuals could have been genotyped at more loci (with higher levels of polymorphism) significant differences may have been detected. It is important to note that the initial Allelic diversity of the study populations was low in comparison to home range populations (Zane *et al.* 1999; Spencer *et al.* 2000) and this reflects a history of bottlenecks. In experimental studies of bottlenecks with *G. affinis* populations founded by just two individuals, the allelic diversity was reduced to 3.5-3.6 from ~15 in the home range population (Spencer *et al.* 2000). Both these values are higher than the initial pre-eradication A of both of the populations in this study. Such low initial diversity undoubtedly reduced our ability to detect significant reductions in A after eradication, as past research has demonstrated that the ability to detect bottleneck signatures decreases with reduced allelic diversity (Spencer *et al.* 2000).

The observed deviations from Hardy-Weinberg expectations in the populations could be due to the presence of null alleles, the Wahlund effect, non-random sampling, or violations of Hardy-Weinberg assumptions (e.g. inbreeding). The deviations from Hardy-

Weinberg are unlikely to represent a Wahlund effect because the individuals that were sampled were from a limited geographical area and might reasonably be expected to be from a random mating population. The presence of null alleles could have been a contributing factor particularly since some of the loci were designed for use with *G. affinis*, a close relative of *G. holbrooki*. Using primers developed from other species can increase the chance of encountering null alleles (Pemberton *et al.* 1995).

In conclusion, this study demonstrates the ability of *Gambusia* populations to avoid the negative effects of bottleneck events that dramatically decrease population size. It is unlikely that the long term viability of either of the study populations will be affected by the eradication attempts, particularly considering the negligible effect on population genetic diversity that was observed. However, it must be noted here that the power to detect any genetic changes in this study was low due to small number of loci used. Both populations have obviously undergone significant bottlenecks in the past as evidenced by their low initial *A*. Despite this low diversity the populations are remarkably robust and can recover rapidly from very severe bottlenecks imposed during eradication. On this basis, the prospects of eradicating *Gambusia,* even from small confined waterbodies, would appear unlikely. From a management perspective, this study reinforces that it is extremely difficult to eradicate fish species once they have become established. However, many practical skills were learned through this study which may help any further eradication attempts. Furthermore, since this study was completed *Gambusia* has been successfully eradicated from both of the study sites using similar methods (*pers. comm.* Grant Scurr). The eventual successful eradication of *Gambusia* was aided by the un-seasonally dry weather of the last couple of years that saw both dams almost dry completely due to the lack of rainfall. In addition to the above successes, there have been

several successful *Gambusia* eradications conducted on mainland Australia (Milner 2006) and in New Zealand (Chadderton *et al.* 2001). Therefore, targeted eradications of source or donor populations should be attempted in suitable locations in an effort to slow the spread of the species. In such cases, every effort should be taken to ensure all individuals in the population are eradicated.

4.5 References

Arthington A. H. (1990) The role of habitat disturbance in the establishment of introduced warm-water fishes in Australia. In 'Introduced and translocated fishes and their ecological effects'. (Ed. DA Pollard) pp. 61-66. (Australian Government Publishing Service: Canberra Bureau of Rural resources proceedings).

Arthington A. H. and Lloyd L. N. (1989) Introduced Poeciliids in Australia and New Zealand. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 333-348. (Prentice Hall: New Jersey).

Bax N. J. and McEnnulty F. (2001) 'Rapid response options for managing marine pest incursions.' CSIRO Marine Research, 21249, Hobart, Australia.

Brown K. L. (1987) Colonization by mosquitofish (*Gambusia affinis*) of a Great Plains river basin. *Copeia* **2**, 336-351.

Chadderton L., Kelleher S., Brow A., Shaw T., Studholme B. and Barrier R. (2001) Testing the efficacy of rotenone as a piscicide for New Zealand pest fish species. In 'Managing invasive freshwater fish in New Zealand'. Hamilton pp. 113-130. (Department of Conservation, New Zealand).

Clair T. A. and Hindar A. (2005) Liming for the mitigation of acid rain effects in freshwaters: A review of recent results. *Environmental Reviews* **13**, 91-128.

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Crooks J. A. and Soule M. E. (1999) Lag times in population explosions of invasive species: causes and implications. In 'Invasive species and biodiversity management'. (Eds OT Sandlund, PJ Schei and A Viken) pp. 103-126. (Kluwer Academic Publishers).

Ehler L. E. (1998) Invasion biology and biological control. *Biological Control* **13**, 127-133.

Foster D. N., Nittim R. and Walker J. (1986) 'Tamar River siltation study.' University of New South Wales, 85/07, Manly Vale.

Hamer A. J., Lane S. J. and Mahony M. J. (2002) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* **132**, 445-452.

Hanski I. (1999) 'Metapopulation Ecology.'pgs. (Oxford University Press: Oxford, U.K.).

Howe E., Howe C., Lim R. and Burchett M. (1997) Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine & Freshwater Research* **48**, 425-434.

Hurlbert S. H. and Mulla M. S. (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* **83**, 125-151.

Ivanstoff W. and Aarn (1999) Detection of predation on Australian native fishes by *Gambusia holbrooki*. *Marine and Freshwater Research* **50**, 467-468.

Kats L. B. and Ferrer R. P. (2003) Alien predators and amphibian decline: review of two decades of science and the transition to conservation. *Diversity and Distributions* **9**, 99-110.

Keane J. P. and Neira F. J. (2004) First record of mosquitofish, *Gambusia holbrooki*, in Tasmania: stock structure and reproductive biology. *New Zealand Journal of Marine and Freshwater Research* **38**, 857-868.

Koya Y. and Kamiya E. (2000) Environmental Regulation of Annual Reproductive Cycle in the Mosquitofish, *Gambusia affinis*. *Journal of Experimental Zoology* **286**, 204-211.

Leberg P. L. (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* **46**, 477-494.

Leberg P. L. (2002) Estimating allelic richness: Effects of sample size and bottlenecks. *Molecular Ecology* **11**, 2445-2449.

Lewis P. O. and Zaykin D. (2001) 'Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0.'pgs. (Free program distributed over the internet. http://lewis.eeb.uconn.edu/lewishome/software.html).

Lloyd L. (1990a) Ecological interactions of *Gambusia holbrooki* with Australian native fish. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 94-97. (Austalian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. (1990b) Native fishes as alternative to the exotic fish, *Gambusia*, for insect control. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 115-122. (Australian Government Publishing Service, Canberra: Magnetic Island, Townsville).

McEnnulty F. R., Bax N. J., Schaffelke B. and Campbell M. L. (2001) 'A review of rapid response options for the control of ABWMAC listed introduced marine pest species and related taxa in Australian waters.' Centre for Research on Introduced Marine Pests, CSIRO Marine Research, 23, Hobart.

Milner R. (2006) '*Gambusia holbrooki*, a management guide and workbook.' Wildcare and NRM North, Launceston, Tasmania.

Morrison B. R. S. (1977) The effects of rotenone on the invertebrate fauna of three hill streams in Scotland. *Fisheries Management* **8**, 128-139.

Myers J. H., Simberloff D., Kuris A. M. and Carey J. R. (2000) Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* **15**, 316-320.

Nei M., Maruyama T. and Chakraborty R. (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1-10.

Oosterhout C. V., Hutchinson W. F., Wills D. P. M. and Shipley P. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Peakall R. and Smouse P. E. (2005) 'GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research.'pgs. (The Australian National University. http://www.anu.edu.au/bozo/GenAlEx: Canberra, Australia).

Peel D., Ovenden J. R. and Peel S. L. (2004) 'NeEstimator: software for estimating effective population size. Version 1.3. Department of Primary Industries and Fisheries. Queensland Government. (Ver. Version 1.3). (Queensland Government, Department of Primary Industries and Fisheries: Brisbane).

Pemberton M., Slate J., Bancroft D. R. and Barrett J. A. (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage or population studies. *Molecular Ecology* **4**, 249-252.

Pulliam H. R. (1988) Sources, sinks and population regulation. *American Naturalist* **132**, 652-661.

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki. Reviews in Fish Biology and Fisheries* **15**, 339-365.

Rivas L. R. (1963) Subgenera and species groups in the poeciliid fish genus *Gambusia* Poey. *Copeia* **1963**, 331-347.

Robbins L. W., Hartman G. D. and Smith M. H. (1987) Dispersal, reproductive strategies, and the maintenance of genetic variability in mosquitofish (*Gambusia affinis*). *Copeia* **1**, 156-164.

Spencer C. C., Chlan C. A., Neigel J. E., Scribner K. T., Wooten M. C. and Leberg P. L. (1999) Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis. Molecular Ecology* **8**, 157-168.

Spencer C. C., Neigel E. J. and Leberg P. L. (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology* **9**, 1517-1528.

Truett G. E., Heeger P., Mynatt R. L., Truett A. A., Walker J. A. and Warman M. L. (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques* **29**, 52-54.

Waples R. S. (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**, 379-391.

Webb C. and Joss J. (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist* **30**, 316-326.

Wilson F. (1960) 'A review of the biological control of insects and weeds in Australia and Australian New Guinea.' 102 pgs. (Commonwealth Agricultural Bureaux: Bucks, England).

Wooten M. C., Scribner K. T. and Smith M. H. (1988) Genetic variability and systematics of *Gambusia* in the southeastern United States. *Copeia* **2**, 283-289.

Zane L., Nelson W. S., Jones A. G. and Avise J. C. (1999) Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* **12**, 61-69.

CHAPTER 5:

Site-based risk assessment protocol as a tool for evaluating risk of *Gambusia* invasion of the Tamar Estuary

5.1 Introduction

Non-indigenous and/or invasive species have become an increasing concern in recent decades, both ecologically and economically, as a result of increased accidental introductions due to global trade (Ricciardi and Rasmussen 1998; Pimentel *et al.* 2000). Approximately 10-30% of introduced species become pests and cause major environmental or economic impacts (Williamson and Fitter 1996; Pimentel *et al.* 2000). In freshwater environments for example, it is generally recognized that non-native species pose significant ecological threats to biodiversity in recipient habitats (Sala *et. al.* 2000; Lodge 2001; Copp *et al.* 2005a). In Australia, the known number of freshwater fish introductions has increased from 8 in 1967, to 43 in 2004 (Koehn and MacKenzie 2004). Species of particular concern are those with the greatest environmental and economic impacts such as brown trout, carp, tilapia, rainbow trout and mosquitofish (Koehn and MacKenzie 2004).

This study focuses on the recent introduction of the mosquitofish, *Gambusia holbrooki*, in the Tamar Estuary, northern Tasmania. *Gambusia holbrooki* and *G. affinis,* collectively known as mosquitofish, are recognized to have a range of negative impacts on native species when they have been introduced to combat mosquitoes (Courtenay and Meffe 1989; N.S.W. Parks and Wildlife Service 2003). These species have become widely distributed throughout mainland Australia, since they were first introduced in the early 1900's (Wilson 1960). They were not introduced into the island state of Tasmania until 1992, when approximately 50 *G. holbrooki* were intentionally and illegally released into a farm dam in the north of the state (IFS *unpublished data*). Since the initial introduction, the species has spread naturally throughout the upper reaches of the Tamar estuary despite

containment and eradication efforts by IFS and stakeholder groups (see Chapters 2 and 4 of this study).

The primary aim of this chapter is to develop a risk assessment protocol that can be used as a management tool to identify sites at risk of *Gambusia* invasion in the Tamar Estuary. For the purposes of this study, the site-specific risk of *Gambusia* invasion incorporates each of the typical phases of invasion; introduction, establishment, spread and impact. The entry or introduction phase consists of the arrival of the non-indigenous species in the new environment. In the establishment phase the invading species has escaped the immediate danger of local extinction and is reproducing in the new environment (Anderson *et al.* 2004a). The species disperses from the initial entry point and begins to occupy available habitat within the new environment, constituting the spread phase (Anderson *et al.* 2004a). The final phase, impact, is characterized by persistence of the newly established species and competition with native species in the new range (Anderson *et al.* 2004b). In this study, as introduction has already occurred and the invasion is currently in a lag phase (*see* Chapter 2); the risk of further dispersal, establishment and impact will be assessed in order to evaluate the future risk of *Gambusia* to the upper Tamar Estuary.

Risk Assessment (RA) is the process of characterizing the likelihood and severity of potentially adverse effects of exposure to hazardous agents or activities (i.e. stressors)(Anderson *et al.* 2004b). Although risk assessment has mainly been utilized to assess the likely impacts of chemicals and other abiotic stressors, it has increasingly been used for assessment of biotic stressors such as non-indigenous species (Landis 2003). The purpose of RA in an invasive species context is to determine which species will be introduced, and which will have adverse impacts, in order to allocate resource,

management and control efforts (Ricciardi and Rasmussen 1998; Colnar and Landis 2007). Protocols for RA and hazard identification have been developed for assessment of non-native freshwater fishes in a number of countries to aid in management of many introductions (Kohler and Stanley 1984; Kahn *et al.* 1999; Kolar and Lodge 2002; Copp *et al.* 2005a). These RA's compare a number of species and generally consist of a large scale climate matching section together with a biology/ecology section where species traits such as physiological tolerance, reproduction, feeding and dispersal mechanisms are assessed. Risk scores are derived from answers provided by experts in the field and relevant published literature (Kohler and Stanley 1984; Kahn *et al.* 1999; Kolar and Lodge 2002; Copp *et al.* 2005a).

In this chapter, a RA procedure is formulated to identify high risk sites for *Gambusia* invasion in the Tamar Estuary. Unlike traditional RA, the protocol outlined here assesses the risk of invasion of only one species (*G. holbrooki*), in one region (Tamar Estuary). The physical, ecological, biological and water chemistry attributes of the individual sites determine their risk ratings and susceptibility to *Gambusia* invasion. This approach operates at a smaller regional scale than traditional RA methods (outlined above) and does not attempt to estimate risk of future new introductions of *Gambusia,* but rather focuses on the risk of further spread of the species in the recipient area. The RA presented here is primarily intended to be used as a management tool for field surveys to aid in identifying sites at high risk of *Gambusia* invasion in the Tamar Estuary. However, with minimal modification and a similar site assessment approach, it could be used in any other region/environment, if and when new introductions of *Gambusia* occur.

5.2 Materials and methods

5.2.1 Habitat assessments and data analysis

The RA was developed from a data set derived from a survey of 27 sites within and around the current distribution of *Gambusia* in the Tamar Estuary (Fig. 5.1). Site data was collected at three random points along a 100m transect along streams and channels and around the perimeter of impoundments. Biotic and physiochemical data were obtained on one occasion while fish species presence was determined using various methods depending on the nature of the site (see Table 5.1). A variety of methods including collapsible baited nets, electrofishing and dip-netting were used during surveys. As fish survey effort varied, only species presence or absence was recorded. Photographs were taken of each site and transect as an extra record of the site characteristics. The mix of categorical and continuous variables included: 1) physical factors such as bank-full width, substrate type and artificial features; 2) biotic factors such as presence of other fish; 3) water chemistry variables e.g. pH, salinity; and 4) large scale factors including total area, site type and proximity to other sites occupied by *Gambusia* (Table 5.1).

Forward stepwise logistic regression (carried out using SPSS ver 14.0) was used to reduce the 19 variables to a smaller set of significant variables that were associated with *Gambusia* presence or absence. When all 19 variables were included in the logistic procedure the resulting model was unstable and none of the variables were significantly correlated with *Gambusia* presence. However, when only variables relating to establishment of *Gambusia* were included (see Table 5.1), coarse substrate was negatively associated with *Gambusia* presence ($P<0.05$). Coarse substrates occur in streams that are

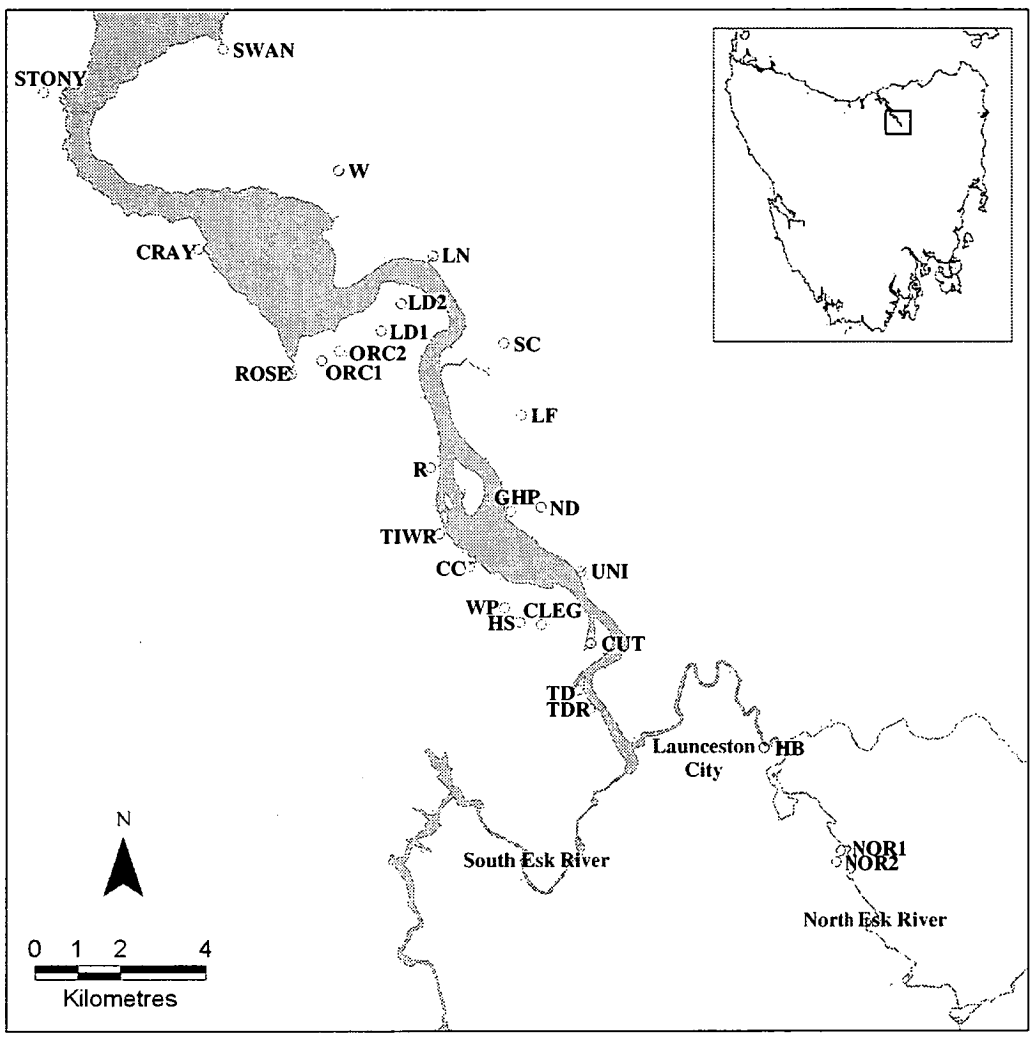

Figure 5.1. Map of the upper Tamar Estuary and surrounding region showing the location of 27 sites surveyed to assess the risks associated with *Gambusia* invasion. Site name codes and general descriptions are given in Appendix 5.2.

Table 5.1. Biotic, physical and chemical factors that were used to construct the risk assessment protocol for *Gambusia* invasion of the Tamar Estuary. Shaded variables were applied to *Gambusia* presence/absence model using logistic regression.

Variable	Type	Units	Methodology
Site type	Categorical	Wetland, Dam, Drainage channel, Tidal creek	Visual
Total area	Continuous	Km^2	Calculated with GIS
Proximity to a site with *Gambusia*	Continuous	Measured in Km via the estuary	Calculated with GIS
Landuse impacts	Categorical	Commercial, forestry, grazing, recreational, residential, natural, other	Visual
Artificial features	Categorical	Bridge, culvert, drain, ford, weir, channel straightening, other	Visual
Tidal connection	Categorical	Present/Absent	Visual
Barriers to dispersal	Categorical	Weir, waterfall, other	Visual
Public Access	Categorical	Present/Absent	Visual
Gambusia	Categorical	Present/Absent	Dipnetting, electrofishing, traps
Fish diversity	Continuous	# species present	Dipnetting, electrofishing, traps
Temperature	Continuous	^{o}C	Electronic probe
Dissolved Oxygen	Continuous	$\mu g\ l^{-1}$	Electronic probe
pH	Continuous		Electronic probe
Salinity	Continuous	ppt	Handheld refractometer
Turbidity	Categorical	Clear, slight, turbid, opaque	Visual
Shaded water	Continuous	% cover	Visual
Macrophytes	Continuous	% cover	Visual
Bankfull Width	Continuous	metres	Tape measure
Substrate type			
- Fine (clay, silt, mud)	Continuous	% of bed area	Visual
- Intermediate size (sand, gravel)	Continuous	% of bed area	Visual
- Coarse (cobble, boulder, bedrock)	Continuous	% of bed area	Visual

subject to fast water flow that carries away fine grained sediments (Richards 2004). Therefore, it can be predicted that sites in the Tamar region with high water flow rates are unlikely to be permanently colonized by *Gambusia*. Substrate type was included in the establishment section of the RA in Q#2.00 and given a high weighting (+5) due to logistic analysis highlighting it as an important variable in predicting *Gambusia* presence or absence (see Appendix 5.1).

5.2.2 Risk assessment protocol

The basis of the risk assessment is the answers to 19 questions (Appendix 5.1) based on the three of the four main phases of an invasion: dispersal, establishment, and impact (risk of new introductions is not assessed). These are combined into a scoring system, with variable weighting, dependent upon evidence in the literature and data collected in this study (Appendix 5.1). The scores of each section are summed to derive a total risk score for each site. A detailed explanation of each question and its scoring system is given in Appendix 5.1.

Dispersal (Section 1) encompasses the potential for the spread of *Gambusia* into a site via natural, deliberate and unintentional pathways from other populations that have already become established in the Tamar region. Six questions in the risk assessment (Section 2) relate to the risk of *Gambusia* establishment at the site in question. Although *Gambusia* have broad range of physiological tolerances (Pyke 2005) and occur in a range of habitats (Lloyd *et al.* 1986) , they do prefer certain habitat attributes (Pyke 2005). These are addressed specifically in Section 2. Section 3 assesses the potential impacts (economic and environmental) of *Gambusia* at the site. Land use, native species and possibility of

eradication are considered in this section.

Answers to all the questions provide a maximum potential score of 39. The total score for each site evaluated in the risk assessment is intended to provide a relative estimate of the sites susceptibility to *Gambusia* invasion.

5.2.3 Validation of the RA

The same 27 sites used to construct the protocol were utilized to evaluate the performance of the RA. However, it was assumed that all sites had not yet been invaded by *Gambusia* although several already support established populations of the fish (see Chapter 2 for details). If the model results in a high score for sites where *Gambusia* populations have already established, then it can be considered to effectively estimate *Gambusia* risk.

5.3 Results

5.3.1 Risk Assessment

A total risk score of ≥30 was achieved at only three of the 27 sites in the Tamar region (Fig. 5.3; Table 5.2). These three sites (TIWR, W and LF) currently support large populations of *Gambusia* (see Chapter *2*, Table 2.1). Over 85% of sites had a range of risk scores from 1-20, while nearly 15% received a risk score ≥21 (Table 5.2). The sites where *Gambusia* has failed to establish all returned scores ≤ 20, as did those where the fish has never been encountered (Table 5.2). Three sites (SC, LD2 and CUT) that support *Gambusia*

populations returned risk scores ≤ 20.

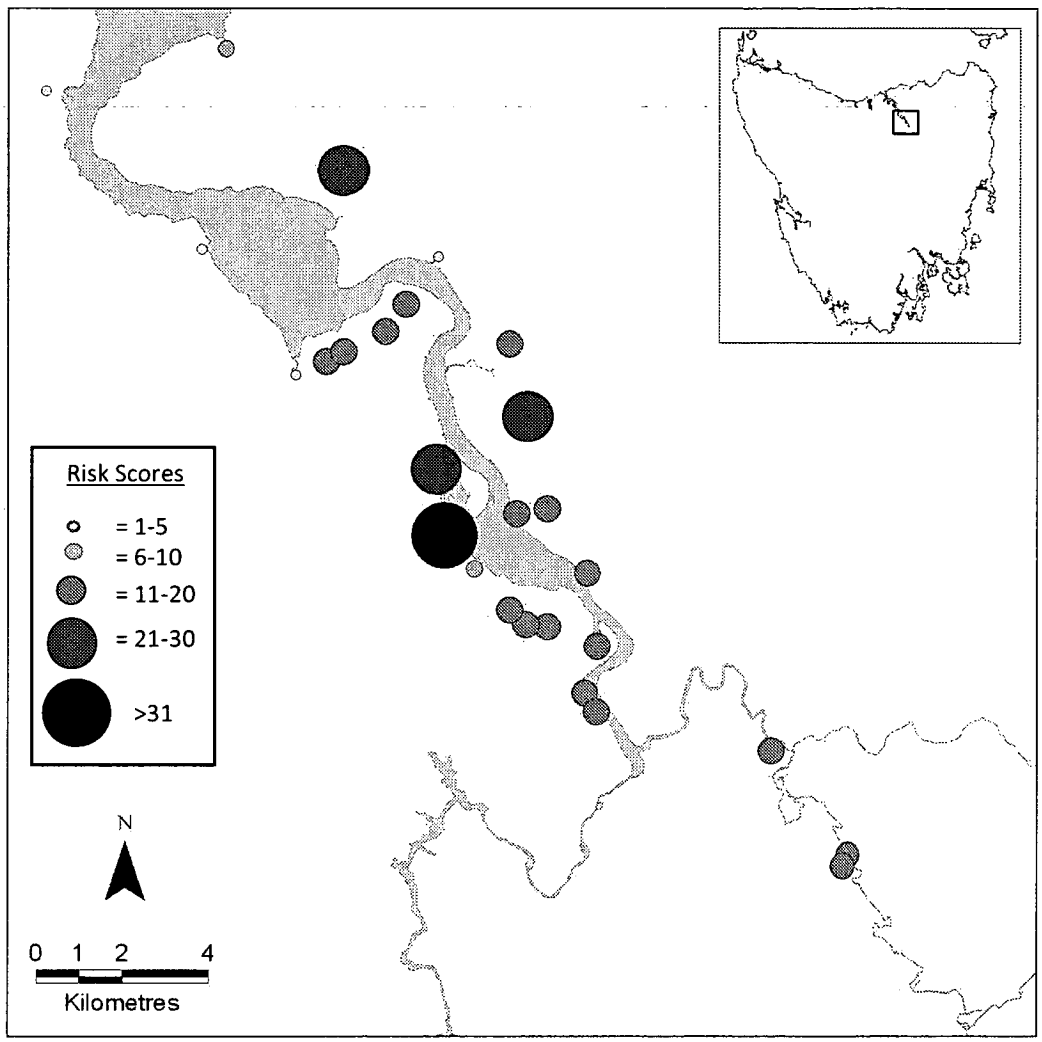

Figure 5.2. *Gambusia* risk assessment scores for 27 sites in the Tamar Estuary. Circle diameter is proportional to risk value.

Table 5.2. Risk assessment results for 27 sites in the Tamar Estuary. Question numbers are in the left column and are divided into three categories; dispersal, establishment, and impact. Sites with dark shading currently support populations of *Gambusia*. Light shading indicates sites where *Gambusia* have been found, but were unable to establish. *Gambusia* have never been encountered at sites with no shading. See Appendix 5.2 for site name codes, and Appendix 5.1 for RA questions.

	Qu.	TIWR	W	LF	R	SC	LD2	CUT	GHP	LD1	CC	LN	ND	HS	CLEG	TD	HB	ORC1	WP	NOR1	NOR2	ORC2	TDR	UNI	SWAN	ROSE	STONY	CRAY
Dispersal	1.00	1	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1
	1.01	1	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	0
	1.02	2	1	1	1	1	2	1	2	2	2	2	2	2	2	2	1	2	2	1	1	2	2	2	2	2	2	2
	1.03	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
	1.04	0	1	1	0	1	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
	1.05	1	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.06	5	5	5	5	5	-5	5	5	-5	5	5	-5	-5	-5	-5	5	-5	5	-5	-5	-5	5	5	5	5	5	5
	1.07						1			0			1	0	1	1		0		1	1	0						
	1.08	2	1	0	2	2		2	2		1	1					1		1				1	1	1	1	1	1
	1.09	2	1	2	2	2		1	1		2	1					0		1				1	0	0	1	1	1
	Sub	14	10	10	11	11	0	10	11	-1	13	12	1	0	1	1	9	-2	11	-2	-2	-2	11	10	9	11	12	10
Establishment	2.00	5	5	5	5	5	5	5	5	5	-5	-5	5	5	5	5	5	5	0	5	5	5	0	0	0	-5	-5	-5
	2.01	5	5	5	5	-5	5	-5	-5	5	-5	-5	5	5	5	5	-5	5	-5	5	5	5	-5	-5	-5	-5	-5	-5
	2.02	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0
	2.03	1	1	2	1	1	1	2	1	1	0	1	1	2	1	1	2	1	2	1	1	1	2	2	1	1	0	1
	2.04	1	2	1	1	2	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	2	1	1	1	2	1	1
	2.05	0	0	0	0	0	2	0	0	2	0	0	2	2	2	0	0	0	0	2	2	0	0	0	0	0	0	0
	Sub	13	14	14	13	4	15	4	3	15	-9	-8	15	15	15	13	4	14	-1	14	14	13	-1	-1	-2	-7	-9	-8
Impact	3.00	2	2	2	2	2	1	0	0	0	0	0	2	1	2	0	0	2	0	1	1	2	0	0	1	0	1	0
	3.01	2	2	2	2	1	2	0	2	1	1	0	2	2	0	2	1	0	2	0	0	0	0	1	1	0	0	1
	3.02	2	2	2	0	2	0	1	2	0	1	1	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	1
	Sub	6	6	6	4	5	3	1	4	1	2	1	4	3	2	2	2	2	3	1	1	2	1	2	3	1	1	2
	Tot	33	30	30	28	20	18	15	18	15	6	5	20	18	18	16	15	14	13	13	13	13	11	11	10	5	4	4

5.4 Discussion

5.4.1 RA performance

Risk assessments are often validated or tested by comparing the results from a number of experts in the field (Pheloung *et al.* 1999; Emery *et al.* 2003; Copp *et al.* 2005a). In this study however, it was not possible to use this approach due to the smaller scale, site-based RA. However, I was able to use known study sites to compare how closely the RA mirrors the actual situation. The results of the site-based RA developed here to estimate *Gambusia* risk in the Tamar Estuary generally reflects the actual situation on the ground. That is, sites where *Gambusia* have established large reproducing populations (e.g. TIWR, LF, W) yielded the highest scores in the RA; while in sites where *Gambusia* have never been encountered, or where they have been found but failed to establish, returned scores of ≤20.

Pheloung *et. al.* (1999) developed a weed risk assessment model for use as a biosecurity tool in evaluating plant introductions. They tested the model against experts' scores of 370 taxa present in Australia. The model was judged on its ability to correctly reject weeds, and accept non weeds, with a small proportion that could not be categorized. The model rejected or accepted over 70% of taxa. All taxa classified as serious weeds and minor weeds were rejected, while only 7% of non-weeds were rejected. This type of RA could be used as a screening tool in any region of the world and is extremely useful for species where scientific knowledge and information is lacking (Pheloung *et. al.* 1999). Similarly, the RA presented in this study is a useful tool as it generally reflects the actual situation on the ground and could be used to predict other

sites at risk of *Gambusia* invasion.

One interesting, but not unexpected result of the RA was that SC, LD2 and CUT yielded relatively low scores (≤20) even though *Gambusia* have been found at all of these sites. LD2 supports a particularly large population of the fish and was the site of initial introduction (Chapter 2). The low RA score was achieved because there is no tidal connection with the estuary and fish are unable to naturally colonize the water body (except in flood conditions). However, the habitat does suit establishment of the fish, and once artificially introduced they were able to establish. This result highlights the fact that the RA cannot predict new introductions facilitated by human activity. In order to incorporate the risk of new introductions occurring into the RA, all possible pathways into the State would need to be fully investigated. As *Gambusia* are a controlled fish and not an aquarium species (see IFS web site) in Tasmania, the most likely introduction pathway is by deliberate illegal means rather than accidental transfer. Unfortunately, estimating and incorporating this type of risk into the RA is problematic.

In the case of site SC, fish were collected in successive years at the site, albeit in small numbers (see Table 2.1 in Chapter 2). The characteristics of the SC site are not particularly suited to the establishment of *Gambusia* (see low establishment score in RA) but the tidal connection ensures the site can receive new dispersers from the nearby LF site. Fish were only ever found at site CUT in one isolated pond created over the summer on a single occasion (Table 2.1 in Chapter 2). Similar to site SC, site CUT is tidally connected to the estuary and able to freely receive dispersers although it is generally unsuitable for establishment of *Gambusia*. This was reflected in the low RA score.

5.4.2 Management significance

Risk assessments of non-indigenous species are primarily intended to be used as a management tool for authorities and organizations charged with preventing new incursions, or implementing control or eradications of non-indigenous species (Pheloung *et al.* 1999; Clunie *et al.* 2002; Harrison and Congdon 2002; Bomford and Glover 2004; Hart Hayes and Landis 2004; Copp *et al.* 2005a). The RA presented here was developed for a similar purpose, with the emphasis on identifying at-risk sites along the Tamar Estuary. The RA indicates that sites where a score < 20 is achieved can be considered at low risk from *Gambusia*. In contrast, sites with relatively high scores (e.g. TIWR, LF, R and W) should be the focus of management efforts as these sites are suitable for *Gambusia* establishment and because they have economic and ecological values that are at risk of being impacted with the establishment of *Gambusia*. The site TIWR has been identified as having prime importance (above other sites) in the spread of *Gambusia* in the Tamar Estuary (Chapter 3), and as *Gambusia* are well established at all of the above mentioned sites (Chapter 2), efforts should be made to prevent the dispersal of fish to and from these sites.

Managers of *Gambusia* in Tasmania could use this RA as a quick way to assess any new survey sites in the Tamar region, and to guide monitoring considerations in the future. As mentioned earlier, in its current format the RA is unable to predict where new introductions are most likely to occur. However, with minor modifications the RA could be used in other parts of the State if there are new introductions or to identify high risk regions. By excluding the section on natural dispersal (Q 1.06-1.09), which specifically relates to the estuarine environment of the Tamar, the RA would still be a good indicator

of regional *Gambusia* risk. The central highland lakes of Tasmania would likely score highly in the RA due to their many ecological and economic values such as endemic fish and frogs, and the recreational trout fishery. The area is at risk of non-indigenous species invasions because it is highly accessible to the general public and is used for a variety of recreational pursuits such as fishing, bushwalking and camping. Public education/awareness signs could be erected in areas identified to be at high risk using a modified RA. Research and management effort should focus on this area in the future.

5.5 Appendices

Appendix 5.1

Site based risk assessment protocol as a tool for evaluating risk of *Gambusia* infestation of the Tamar estuary.

Assessment of *Gambusia* risk can be divided in to three main categories: Dispersal, Establishment, and Impact.

1. Dispersal

Gambusia can spread and disperse in the Tamar estuary in three possible ways; via deliberate new introductions, by unintentional transfer (human or bird mediated) of the current distribution and by natural spread (via the estuary) of the current distribution. The risk of a deliberate unauthorized importation will not be estimated in this assessment. Of primary concern is the continued spread of the existing populations already established in the estuary.

The threat of deliberate or unintentional spread is considered to be low because the general public are unaware of the location of all but one *Gambusia* population (TIWR), and public awareness campaigns highlighting impacts and the importance of limiting the spread of the fish are ongoing.

Deliberate

These questions are intended to assess the risk of deliberate transfers of *Gambusia* to a new site from an existing population.

Q 1.00 Is the site easily accessible by the general public?
Explanation: Fish transfers are more likely to occur at sites that are accessible to the general public (Copp *et al.* 2005b). Therefore, sites on private property are assigned a lower risk score than those on public land.
Scoring: Yes = +1, No = 0.

Q 1.01 Is this site close to a residential area?
Explanation: Unauthorized fish introductions are more likely to occur close to residential areas (Copp *et al.* 2005b).
Scoring: if the site is within walking distance (<1km) of a residential area score = +1, if > 1km from a residential area score = 0.

Q 1.02 What is the distance to the nearest public road?
Explanation: Water bodies close to public roads are at increased risk of unauthorized fish introductions because this increases their accessibility. The risk decreases as the distance from a road increases (Copp *et al.* 2005b).
Scoring: if the site is <100 from a public road score = +2, if the distance is >100m and <1km score = +1 and if the distance is >1km then score = 0.

Proceed to Q 1.03

Unintentional

These questions identify possible pathways for unintentional transfer of *Gambusia* throughout the Tamar estuary.

Q 1.03 Is this site popular with recreational anglers?

Explanation: If the site is popular with recreational anglers there is an increased risk of unintentional introduction of fish via bait or movement of nets and other equipment (Copp *et al.* 2005a).
Scoring: Yes = +1, No = 0.

Q 1.04 Does water extraction occur at the site?
Explanation: If water is artificially moved into or out of the site then the risk of unintentional transfer of fish is increased.
Scoring: Yes = +1, No = 0.

Q 1.05 Does the site have an obvious and large resident water bird community?
Explanation: Birds can potentially transfer fish from one site to another by carrying mud on their feet or by regurgitation. If the site has a large water bird community then the risk of introduction of *Gambusia* is increased.
Scoring: Yes = +1, No = 0.

Natural

Questions in this section are given more weight because the spread of *Gambusia* by natural means is far more likely to occur than by other pathways (above). Significant natural spread (<2km/year) has been documented (Chapter 2) in the estuary since the initial introduction.

Q 1.06 Is the site tidally connected to the estuary?
Explanation: The risk of natural introduction of fish is much higher in sites that have a permanent tidal connection to the estuary. Sites with no tidal connection present a permanent physical barrier to fish dispersal.
Scoring: Yes = +5 proceed to question 1.08, No = -5 proceed to next question.

Q 1.07 Could the site become connected with the main estuary during a flood?
Explanation: This is a low probability risk, however flooding of the Tamar valley can occur when periods of intense rainfall coincide with high tides. The answer to this question depends on the knowledge of the height of physical barriers and elevation of the site in question.
Scoring: No, low chance of becoming connected = 0, Yes, high = +1

Q 1.08 Are there barriers to fish dispersal at the site?
Explanation: Even if the site is tidally connected to the estuary there may be barriers to fish dispersal within the site preventing further spread, such as weirs, levees, and waterfalls.
Scoring: if Yes, 1 barrier, score = +1, If >1 barrier score = 0, No = +2.

Q 1.09 What is the distance (via the estuary) to the nearest *Gambusia* infested site?
Explanation: Sites in close proximity to established *Gambusia* populations are more likely to be invaded than sites a greater distance away.
Scoring: <2km from a Gambusia population score = 2, 2-10km score = 1, and >10 km score = 0.

2. Establishment

Gambusia will be able to establish populations at sites in the Tamar estuary where its habitat requirements are satisfied. Although *Gambusia* are generally hardy and can tolerate a wide range of environmental conditions they have a preference for certain conditions. These risks will be assessed under the Habitat subheading. The questions in the second section (Stressors) relate to processes or factors other than habitat that can affect the risk of establishment by *Gambusia*.

Habitat

Q 2.00 What is the dominant substrate type at the site?

Explanation: *Gambusia* are known to prefer still or slow flowing water (Pyke 2005). Substrates composed of fine silt or mud in aquatic habitats reflect little or no flow of water. Logistic regression of several habitat parameters identified substrate composition of prime importance in determining establishment of *Gambusia* at sites in the Tamar estuary ($P<0.05$).
Scoring: coarse substrate = -5, intermediate = 0, fine = +5

Q 2.01 Is there a still backwater area of the habitat?
Explanation: Even though *Gambusia* prefer still water they may be able to establish in sites with water movement if there is a

backwater area that can be utilized as a refuge (Pyke 2005).
Scoring: No = -5, Yes = +5

Q 2.02 Are there macrophytes at the site?
Explanation: *Gambusia* use macrophytes to provide shelter from predation and to forage for food (Pyke 2005). In sites where macrophytes are not abundant, *Gambusia* would be more susceptible to predation by native species and therefore less likely to establish.
Scoring: Yes = 1, No = 0

Stressors

In this section we look at other factors or stressors that can affect invasion by *Gambusia*.

Q 2.03 Are there any obvious physical modifications, pollution and/or human disturbance to the site?
Explanation: these types of impacts often severely effect native species but *Gambusia* are extremely resistant to these types of impacts and can often outcompete in modified habitats (Arthington and Milton 1983). Physical modifications includes channel straightening, weirs, banks and screens.
Scoring: Yes = +1 for each, No = 0

Q 2.04 Are there landuse impacts at the site ie forestry, grazing, commercial, recreational?
Explanation: same as above.
Scoring: Yes = +1 for each, No = 0

Q 2.05 Are there native fish species present at the site?
Explanation: this question assesses the level of potential biotic resistance to *Gambusia* invasion. Native fish species are potential predators and competitors of *Gambusia* that may affect establishment success (Baltz and Moyle 1993).
Scoring: Yes = 0, No = +2

3. Impact

The potential impacts of *Gambusia* in the Tamar Estuary can be assessed by dividing them into economic and environmental impacts.

Economic

Q 3.00 Is there potential for economic losses as a result of *Gambusia* infestation at the site?
Explanation: Grazing could be affected by *Gambusia* if the water body is used to water livestock. *Gambusia* densities can get so high that livestock are unable to get water. If the landuse is recreational and the site is mainly used for tourism to showcase native fauna then the number of users could be affected. Tourism or agricultural sectors may be economically impacted if restrictions are placed on water extraction and water transfer. Recreational fisheries could be affected if important fisheries are invaded by *Gambusia*.
Scoring: Yes, high potential = +2, moderate = +1, Low = 0

Environmental

Q 3.01 Is there potential for negative impacts on native species at the site?
Explanation: *Gambusia* can negatively impact on frog (see Chapter 2), fish and zooplankton species where they have been introduced (Hurlbert and Mulla 1981; Arthington and McKenzie 1997; Webb and Joss 1997; Hamer *et al.* 2002). This question is used to identify if there are species that inhabit the site that may be susceptible to predation or competition by *Gambusia*. Species that have similar niche requirements are most at risk.
Scoring: Yes, high potential = +2, moderate = +1, Low = 0

Q 3.02 Would it be possible to eradicate *Gambusia* from the site?
Explanation: Eradication of *Gambusia* has been attempted at several sites in the Tamar region (Chap. 4). At some sites it would be impossible to eradicate *Gambusia* because of the size and complexity of the habitat, the cost involved, or because re-invasion would occur if tidal connectivity is maintained. There is an economic component to this question because eradication costs can vary widely among sites.
Scoring: Relatively easy & low cost = 0, Intermediate = +1, Difficult & high cost = +2

Appendix 5.2. Site names and brief descriptions of sites used in construction and validation of *Gambusia* risk assessment.

Code	Site name	Description
TIWR	Tamar Island Wetlands Reserve	Wetland
W	Windemere	Wetland
LF	Landfall	Network of drainage channels
R	Riverside	Farm dam
SC	Station creek	Creek with tidal inundation
LD2	Legana Dam 2	Farm dam
CUT	The Cut	Modified river channel
GHP	Green Hillock Point	Tidal swamp
LD1	Legana Dam 1	Farm dam
CC	Cormiston Creek	Tidal creek
LN	Lady Nelson Creek	Tidal creek
ND	Newnham Dam	Farm dam
HS	High School	Dam in Riverside high school grounds
CLEG	Cleghorn Road Dam	Dam adjacent to residential area
TD	Tailrace dam	Recreational dam
HB	Hobblers bridge	Tidal drainage channel
ORC1	Orchard Dam 1	Farm dam
WP	Windsor Park	Tidal creek
NOR1	Norwood dam 1	Farm dam
NOR2	Norwood dam 2	Farm dam
ORC2	Orchard Dam 2	Farm dam
TDR	Tailrace drain	Drainage channel
UNI	University creek	Tidal creek
SWAN	Swan bay creek	Tidal creek
ROSE	Rosevears turnoff	Tidal creek
STONY	Stony River	Tidal creek
CRAY	Craythorne road	Tidal creek

5.6 References

Anderson M. C., Adams H., Hope B. and Powell M. (2004a) Risk analysis for invasive species: General framework and research needs. *Risk Analysis* **24**, 893-900.

Anderson M. C., Adams H., Hope B. and Powell M. (2004b) Risk assessment for invasive species. *Risk analysis* **24**, 787-793.

Arthington A. and McKenzie F. (1997) 'Review of impacts of displaced/introduced fauna associated with inland waters.' Central Queensland University Publishing Unit.

Arthington A. H. and Milton D. A. (1983) Effects of urban development and habitat alterations on the distribution and abundance of native and exotic freshwater fish in the Brisbane region, Queensland. *Australian Journal of Ecology* **8**, 87-101.

Baltz D. M. and Moyle P. B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications* **3**, 246-255.

Bomford M. and Glover J. (2004) 'Risk assessment model for the import and keeping of exotic freshwater and estuarine finfish.' Bureau of Rural Sciences for the Department of Environment and Heritage, Canberra.

Clunie P., Stuart I., *et al.* (2002) 'A risk assessment of the impacts of pest species in the riverine environment in the Murray-Darling Basin.' Department of Natural Resources and Environment, Arthur Rylah Institute for environmental research, Project R2006, Heidelberg, Victoria.

Colnar A. M. and Landis W. G. (2007) Conceptual model development for invasive species and the regional risk assessment case study: The European green crab, *Carcinus maenas*, at Cherry Point, Washington, USA. *Human and Ecological Risk Assessment* **13**, 120-155.

Copp G. H., Garthwaite R. and Gozlan R. E. (2005a) 'Risk identification and assessment of non-native freshwater fishes: concepts and perspectives on protocols for the UK.' CEFAS, 129, Lowestoft.

Copp G. H., Wesley K. J. and Vilizzi L. (2005b) Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. *J. App. Ichthyol* **21**, 263-274.

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Emery R. N., Poole M. C., Botha J. H. and Hardie D. C. (2003) Semi-quantitative model for ranking exotic invertebrate pest threats in Western Australia. In 'Australian postharvest technical conference'. Canberra. (Eds EJ Wright, MC Webb and E Highley) pp. 102-107. (CSIRO, Stored Grain Research Laboratory).

Hamer A. J., Lane S. J. and Mahony M. J. (2002) The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* **132**, 445-452.

Harrison D. A. and Congdon B. C. (2002) 'Wet tropics vertebrate pest risk assessment scheme.' Cooperative research centre for tropical rainforest ecology and management, Cairns.

Hart Hayes E. and Landis W. G. (2004) Regional ecological risk assessment of a near shore marine environment: Cherry Point, WA. *Human and Ecological Risk Assessment* **10**, 299-325.

Hurlbert S. H. and Mulla M. S. (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* **83**, 125-151.

Kahn S. A., Wilson D. W., Perera R. P., Hayder H. and Cerrity S. E. (1999) 'Import risk analysis on live ornamental finfish.' Australian quarantine and inspection service, Canberra.

Koehn J. D. and MacKenzie R. F. (2004) Priority management actions for alien freshwater fish species in Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 457-472.

Kohler C. C. and Stanley J. G. (1984) A suggested protocol for evaluating proposed exotic fish introductions in the United States. In 'Distribution, biology and management of exotic fishes'. (Eds WR Courteney and JR Stauffer) pp. 387-406. (Johns Hopkins University Press: London).

Kolar C. S. and Lodge D. M. (2002) Ecological predictions and risk assessment for alien fishes in North America. *Science* **298**, 1233-1236.

Landis W. G. (2003) Twenty years before and hence; Ecological risk assessment at multiple scales with multiple stressors and multiple endpoints. *Human and Ecological Risk Assessment* **9**.

Lloyd L. N., Arthington A. H. and Milton D. A. (1986) The mosquitofish - a valuable mosquito control agent or a pest? In 'The ecology of exotic plants and animals: some Australian case studies'. (Ed. Kitching). (John Wiley and Sons: Brisbane).

Lodge D. M. (2001) Lakes. In 'Global biodiversity in a changing environment: Scenarios for the 21st century'. (Eds FSI Chapin, OE Sala and E Sanwald) pp. 227-213. (Springer-Verlag: New York).

N. S. W. Parks & Wildlife Service (2003) 'NSW threat abatement plan. Predation by *Gambusia holbrooki* - The plague minnow.' NPWS, Hurstville, NSW.

Pheloung P. C., Williams P. A. and Halloy S. R. (1999) A weed risk assessment model for

use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management* **57**, 239-251.

Pimentel D., Lach L., Zuniga R. and Morrison D. (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience* **50**, 53-65.

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* **15**, 339-365.

Ricciardi A. and Rasmussen J. B. (1998) Predicting the identity and impact of future biological invaders: A priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1759-1765.

Richards K. (2004) 'Rivers, form and process in alluvial channels.' 376 pgs. (Blackburn press: London).

Sala O. E. et. al. (2000) Global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.

Webb C. and Joss J. (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist* **30**, 316-326.

Williamson M. and Fitter A. (1996) The varying success of invaders. *Ecology* **77**, 1661-1666.

Wilson F. (1960) 'A review of the biological control of insects and weeds in Australia and Australian New Guinea.' 102 pgs. (Commonwealth Agricultural Bureaux: Bucks, England).

CHAPTER 6:

Summary

The present investigation is the first comprehensive study of *Gambusia holbrooki* in Tasmania. The study was an attempt to assess the status of the pest fish in the state, its distribution and possible impacts, and to increase our knowledge about the early stages of the invasion process of this fish. In most studies of non-native *Gambusia* populations little is known of the number, timing and origin of the introduced fish, and most introductions occurred many decades before the studies were conducted (Wilson 1960; Arthington and Lloyd 1989). The unique aspect of this study is that it focused on a relatively recent and well documented introduction event allowing for an in depth examination of natural range expansion, meta-population variation, and the effects of dispersal and eradication attempts on the genetic structure of populations.

6.1 Management and future research

In this study, it was determined that *Gambusia* spread naturally at relatively slow rate (see Chapter 2). This finding is somewhat surprising since *Gambusia* are an incredibly widespread fish and are found in every inhabited continent (Krumholz 1948; Lloyd and Tomasov 1985; Arthington and Lloyd 1989). Obviously their success as invaders is not reliant on rapid spread in a new environment. This has also been shown to be the case with other invasive species (Ricciardi and Cohen 2006). Factors such as propagule pressure, and the size and frequency of introduction events are likely to be critical in determining whether the species successfully establishes or not (Lockwood *et al.* 2005; Drake and Lodge 2006). We know that in Australia (and worldwide) *Gambusia* have been deliberately translocated by people in an effort to combat mosquito-borne disease (Wilson 1960). These repeated introductions over a long period of time, coupled with the

fishes advantageous biological, physiological and population attributes (Pyke 2005), are more likely to explain why *Gambusia* have been such successful invaders. The Tasmanian *Gambusia* populations are a result of an illegal deliberate introduction and this study indicates that further natural range expansion and impacts are likely to be slow and minimal within the Tamar Estuary. However, management efforts should focus on restricting the fish to the estuary and on public awareness campaigns aimed at preventing new translocations to other parts of the state.

Past studies have shown that *Gambusia* can have a range of negative impacts in locations where they have been introduced (Courtenay and Meffe 1989; Lloyd 1990; Garcia-Berthou 1999; N. S. W. Parks and Wildlife Service 2003). Similarly, in this study *Gambusia* were found to negatively affect the tadpole community in a small lentic pond (Chapter 2). Tasmanian *Gambusia* populations tended to reach high population densities in these enclosed lentic habitats with dense macrophyte cover, indicating that they are the preferred habitat type of the fish (Chapter 2). These large populations could potentially act as a source for new populations, both via human translocation and by natural means during flood conditions. Therefore, every effort should be made to eradicate these large populations from enclosed water bodies. In this study, although two unsuccessful eradications were attempted and documented, there have been many successful *Gambusia* eradications carried out in Australia, NZ and overseas (Chadderton *et al.* 2001; Milner 2006). Since this study was conducted, further eradications have been carried out at the same two dams (LD2 and R) and it appears that *Gambusia* have been totally eradicated from both locations (Grant Scurr (Tamar NRM) *pers. comm.*). Management efforts should continue in this area and would reduce the risks of further *Gambusia* spread both within and outside of the Tamar Estuary.

Total eradication of *Gambusia* from large areas or regions is generally considered impossible using currently available technology and methods (N.S.W. Parks and Wildlife Service 2003). However, research is currently being undertaken by CSIRO to develop "daughterless" technology where sex ratios are biased towards males (Ron Thresher *per. comm.*). Daughterless technology works by silencing the production of the protein aromatase which stimulates female development (Ron Thresher *per. comm.*). Although the technology has primarily been developed for use with Carp, *Gambusia* have been used as a model species in the research (Ron Thresher *pers. comm.*). This is a long term approach which could be used to control *Gambusia*, carp and other invasive species in the future.

In the short term, research and management efforts should focus on developing and optimizing the efficacy and cost effectiveness of current eradication and control methods. Traditional approaches such as poisoning of fish with toxicants such as rotenone or lime could be refined and optimized. More environmentally friendly approaches such as strategic isolation and drying out of ponds in wetland habitats (as described in Milner 2006) has also been successful in some cases and should be explored further in appropriate habitats. Fish dispersal and access to suitable habitat can be blocked using fine mesh screens over drainage pipes and channel connectors. These measures are not intended to eradicate the fish but may exclude them from ecologically important areas or reduce population densities sufficiently to lower the risk of negative impacts on native species and habitats.

Analysis of the genetic structure of *Gambusia* populations using microsatellite technology showed that Australian populations are characterized by low diversity. Tasmanian fish could have been sourced from Queensland via one introduction event to Tasmania. However, without further research (possibly using another type of marker such

as mitochondrial DNA) this cannot be confirmed.The hyper-variability of microsatellites provides resolution not possible with other technologies (e.g. allozymes) when examining populations with low diversity (Rollins *et al.* 2006). Genetic analysis is beginning to be utilized in more and more studies of invasive animals to identify sources and examine population structure (Rollins *et al.* 2006).

This study confirmed that risk assessment is a valid and useful tool in NIS management. It would never be possible to obtain quantitative data on every aspect of the invasion of a NIS in a new area. Risk assessment allows us to make inferences from the available data and to incorporate other relevant non-quantitative information which directly affects the invasion (Anderson *et al.* 2004). This study showed that RA can be used at a regional scale to highlight sites most at risk from *Gambusia*. Although primarily constructed for use in the Tamar Estuary, the RA could easily be modified for use in other parts of the state. The RA is a holistic approach to invasions and allows for incorporation of scientific and stakeholder group interests. From a management perspective, this research has identified sites most at risk of negative impacts (e.g. TIWR) and efforts can now be focused on these sites. Yearly distribution surveys can be carried out more rapidly that target sites most likely (ie with high risk scores) to be invaded by *Gambusia*.

6.2 Thesis summary

The major findings of this study are summarized below;

1) *G. holbrooki* is not spread rapidly via natural dispersal in the Tamar region, but is

able to negatively affect native species in lentic habitats where it can reach high population densities.

2) *Gambusia* is not able to establish populations in all habitat types in the Tamar Estuary. If the species remains restricted to the region, negative environmental impacts are limited due to minimal overlap with at-risk species. However, as the dispersal of these fish is so often linked to deliberate or accidental human-assisted translocations further spread within Tasmania is likely.

3) Genetic diversity of Australian *G. holbrooki* is low compared to native populations in southern USA.

4) Anecdotal reports of a single introduction event in Tasmania correspond with the pattern of consistent genetic similarity of all Tamar Estuary *Gambusia* populations. Tasmanian populations were genetically similar to the single Queensland population sampled here suggesting the fish could have been sourced from south-east Queensland.

5) TIWR is likely to be the primary population driving the dispersal and range expansion of *Gambusia* in the Tamar Estuary.

6) No single eradication method or toxicant is universally effective in eradicating *Gambusia*. The methods used must take into account site specific characteristics.

7) *Gambusia* populations recover quickly from eradication attempts and their long term viability does not appear to be affected by the subsequent reduced genetic diversity and shifts in allele frequencies.

8) The risk assessment generally reflected the observed distribution of *Gambusia* in the Tamar Estuary and could be used to predict sites most at risk from *Gambusia* introduction, establishment and impacts.

Finally, whilst presenting insights into the ecology and population biology of *G. holbrooki* this study also provides applied research that is of practical use in future management of the pest fish in Tasmania.

6.3 References

Anderson M. C., Adams H., Hope B. and Powell M. (2004) Risk assessment for invasive species. *Risk analysis* **24**, 787-793.

Arthington A. H. and Lloyd L. N. (1989) Introduced Poeciliids in Australia and New Zealand. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 333-348. (Prentice Hall: New Jersey).

Chadderton L., Kelleher S., Brow A., Shaw T., Studholme B. and Barrier R. (2001) Testing the efficacy of rotenone as a piscicide for New Zealand pest fish species. In 'Managing invasive freshwater fish in New Zealand'. Hamilton pp. 113-130. (Department of Conservation, New Zealand).

Courtenay W. R. J. and Meffe G. K. (1989) Small fishes in strange places: A review of introduced Poeciliids. In 'Ecology and Evolution of Livebearing Fishes'. (Eds GK Meffe and FFJ Snelson) pp. 319-331. (Prentice Hall: New Jersey).

Drake J. M. and Lodge D. M. (2006) Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. *Biological Invasions* **8**, 365-375.

Garcia-Berthou E. (1999) Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* **55**, 135-147.

Krumholz L. A. (1948) Reproduction in the western mosquitofish *Gambusia affinis affinis* (Baird and Girard) and its use in mosquito control. *Ecological Monographs* **18**, 1-43.

Lloyd L. (1990) Ecological interactions of *Gambusia holbrooki* with Australian native fish. In 'Introduced and Translocated Fishes and their Ecological Effects'. (Ed. DA Pollard) pp. 94-97. (Austalian Government Publishing Service, Canberra: Magnetic Island, Townsville).

Lloyd L. N. and Tomasov J. F. (1985) Taxonomic status of the mosquitofish, *Gambusia affinis* (Poeciliidae), in Australia. *Aust.J.Mar.Freshwat.Res.* **36**, 447-451.

Lockwood J. L., Cassey P. and Blackburn T. (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* **20**, 223-228.

Milner R. (2006) '*Gambusia holbrooki*, a management guide and workbook.' Wildcare and NRM North, Launceston, Tasmania.

N. S. W. P. & W. Service (2003) 'NSW threat abatement plan. Predation by *Gambusia holbrooki* - The plague minnow.' NPWS, Hurstville, NSW.

Pyke G. H. (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* **15**, 339-365.

Ricciardi A. and Cohen J. (2007) The invasiveness of an introduced species does not

predict its impact. *Biological Invasions.* **9**, 309-315.

Rollins L. A., Woolnough A. P. and Sherwin W. B. (2006) Population genetic tools for pest management: a review. *Wildlife Research* **33**, 251-261.

Wilson F. (1960) 'A review of the biological control of insects and weeds in Australia and Australian New Guinea.' 102 pgs. (Commonwealth Agricultural Bureaux: Bucks, England).